Antioxidant activity of *Vachellia* species, pork quality and fatty acid composition from pigs supplemented with graded levels of Vachellia *tortilis* leaf meal

By

Khanyile Mbongeni

A dissertation submitted in fulfilment of the requirements for the degree of

Doctor of Philosophy in Animal Science

in the

Discipline of Animal and Poultry Science

School of Agricultural, Earth and Environmental Sciences

College of Agriculture, Engineering and Science

University of KwaZulu-Natal

2017

Pietermaritzburg, South Africa

Supervisor: **Professor Michael Chimonyo**

Declaration

I, Mbongeni Khanyile, Student number: 213555045 hereby declare that:

1. The research reported in this dissertation, except where otherwise indicated in the result of my own endeavors conducted under the supervision of Prof. M. Chimonyo;
2. The dissertation has not been submitted for any degrees or examination at any University;
3. All assistance towards production of this work and all references contained herein have been duly acknowledged and
4. Where I have reproduced a publication which I am an author or co-author, I have indicated which part of the publication was contributed by me.

Signed at …….. on the ……...day of ……2017

_____

M. Khanyile

Approved as to style and content by:

_____

Prof M. Chimonyo

(Promoter)

List of Abbreviations

a*	Redness of meat
AA	Antioxidant activity
ADF	Acid detergent fibre
ADFI	Average daily feed intake
ADG	Average daily gain
AI	Atherogenic index
ALA	α-linolenic acid
ANOVA	Analysis of variance
AOAC	Association of Official Agricultural Chemists
ARE	Animal research ethical committee
b*	Yellowness of meat
BCKF	Back fat thickness
BHT	Butylated hydroxytoluene
BSA	Bovine serum albumin
BW	Body weight
CDV	Cardiovascular disease
CIE	Commission International De l ‘Eclairage
CLA	Conjugated linoleic acid
CM	Cold mass
CS	Cooler shrink
CW	Cold dressed weight
DM	Dry matter

DP	Dressing percentage
CP	Crude protein
CS	Cooler shrink
DFD	Dark, dry and firm
DHA	Docosahexaenoic acid
DM	Dry matter
DPA	Docosapentaenoic acid
DPPH	2, 2-diphenyl-1-picryhydrazyl
EDTA	Ethylene diamine tetra acetic acid
EPA	Eicosapentaenoic
FA	Fatty acid
FAME	Fatty acid methyl esters
FRAP	Ferric reducing antioxidant
G: F	Gain: feed
GLM	Generalized linear model
HDL	High-density lipoprotein
LA	Linoleic acid
MUFA	Monounsaturated fatty acids
NDF	Neutral detergent fibre
NRC	National Research Council
NVI	Nutritive value index
PSE	Pale, soft and exudative
RSA	Radical scavenging activity

SAS	Statistical Analysis Systems
SD	Standard deviation
SEM	Standard error of means
SFA	Saturated fatty acids
SDS	Sodium dodecyl sulfate
SW	Slaughter weight
TI	Thrombogenic index
ORR	Oxidation rate ratio
PUFA	Polyunsaturated fatty acids
UKZN	University of KwaZulu-Natal
WBSF	Warner Bratzler Shear Force
WHC	Water holding capacity
WHO	World Health Organization of the United Nations
WM	Warm mass

Abstract

The objectives of the study were to assess antioxidant activity of five selected *Vachellia* species leaves and to determine the effect of Vachellia *tortilis* leaf meal based diet on growth performance, carcass characteristics, pork quality and fatty acid profile. Methanolic extracts from the leaves of *Vachellia xanthophloe, V. robusta, V. tortilis, V. nilotica* and *V. nigrescens* were compared for their protein precipitating capacity and antioxidant activities. *Vachellia tortilis* leaves exhibited high phenolic content with a correspondingly high protein precipitating capacity. Total phenolics ranged from 1.7×10^{-8} mg/mg to 5.89×10^{-7} mg/mg. *Vachellia tortilis* and *V. xanthophloe* exhibited high protein precipitating capacity (54.2 and 52.0 %, respectively). The lowest protein precipitating capacity was recorded for *V. nilotica* (9.2 %). The concentration of an antioxidant which induces a response halfway between the baseline and maximum after a specified exposure time of the extracts (EC_{50}) in the DPPH free radical scavenging assay ranged from 0.570 µg/ml to 0.762 µg/ml, compared to 0.481 µg/ml for ascorbic acid. A dose-depended curvilinear response was obtained for *V. xanthophloe, V. robusta, V. tortilis* and *V. nigrescens* in the ferric-reducing power assay. All extracts, except *V. tortilis* exhibited high antioxidant activity (81.6 to 90.7 %) comparable to butylated hydroxytoluene (BTH) (94.8 %) based on the rate of *β*-carotene bleaching. The oxidation rate ratio (ORR) values of these extracts (0.09 to 0.18) were also comparable to that of BTH (0.08). Following the characterization of the antioxidant activities and protein precipitating of Vachellia species, Vachellia tortilis leaf meal was chosen based on the availability in the study area and as a continuation work documented else whereby Khanyile et al., 2014; Ndou et al., 2015; Khanyile et al., 2016.

Forty-eight Large White x Landrace male pigs (8 pigs/treatment), averaging 63 ± 0.93 (SD) kg BW), randomly allotted to six diets containing 0, 30, 60, 90, 120 and 150 g/kg DM of *V. tortilis* leaf meal. Average feed intake (ADFI), average daily gain (ADG) and gain: feed (G: F) ratio were recorded weekly. These pigs were slaughtered at 18 weeks of age at a commercial abattoir and their carcass characteristics were assessed. Following 24 hours of chilling the carcasses at 2 ºC, *Longissimus dorsi* muscle (LD) samples were taken between the 5^{th} and last 13^{th} rib, vacuum packed in plastic bags, placed on ice in insulated picnic coolers and transported to the laboratory, where they were kept at -20 ºC pending for meat quality analysis. Overall, ADFI increased linearly (Y= 0.061x + 2.155; $P < 0.05$) with inclusion of *V. tortilis.* There was a linear decrease ($P < 0.05$) in ADG and G: F ratio with increasing *V. tortilis* inclusion. The ADG and G: F decreased linearly (Y = -0.00629x + 0.925; Y= -0.0063 + 0.415; $P < 0.05$) with inclusion level of *V. tortilis* leaf meal. The inclusion of *V. tortilis* linearly ($P < 0.05$) decreased slaughter weight, warm carcass weight, cold carcass weight, cooler shrink and back-fat thickness. Dressing percentage, pH at 45 minutes (pH_{45}) and temperature at 45 minute ($temperature_{45}$) were not ($P > 0.05$) affected by *V. tortilis* inclusion.

Ultimate meat pH (pH 24), Hue and Warner Bratzler Shear Force (WBSF), increase quadratically ($P < 0.05$) with increasing levels of *Vachellia tortilis*. There was a quadratic decrease ($P < 0.05$) in intramuscular fat (IMF), pork redness (a^*) with increasing *V. tortilis* in the diet. There was a linear decrease ($P < 0.05$) in pork moisture, lightness (L^*), yellowness (b^*), Chroma and cooking loss, while muscle protein increase linearly ($P <$

0.05) with increasing *V. tortilis* in the diet. Inclusion levels of V. *tortilis* leaf meal did not affect the dry matter percentage and ash of the pork sample. Total fatty acid content, proportions of individual and total saturated fatty acids (SFA), C24:1, C18:2n6 and total n-6 polyunsaturated fatty acids (PUFA), n-6/n-3 ratio, atherogenic index (AI) and thrombogenic index (TI) decreased linearly (P <0.001) with increasing levels of *V. tortilis* leaf meal in the diet. There was a quadratic increase (P <0.001) in monounsaturated fatty acids (MUFA). Total *n*-3 PUFA, total PUFA and nutritive value index increased and then decreased in a quadratic (P <0.001) fashion with increasing inclusion of *V. tortilis* leaf meal. The highest proportions of C18:3*n*-3, C20:5*n*3, C20:3*n*3 and total *n*-3 PUFA were obtained at 70, 65, 62 and 68 g/kg DM of V*. tortilis* inclusion, respectively (P< 0.05). The largest proportion C20:2*n*-6, C20:3n-6 and C22:2*n*-6 was reached at 91, 93 and 67 g/kg DM of *V. tortilis* leaf meal inclusion levels. The maximum total MUFA and total *n*-3 fatty acids were obtained at 69 and 68 g/kg DM of *V. tortilis* leaf meal inclusion level, respectively. It can be concluded that while ADG, ADFI and carcass quality decreased with increase inclusion level of leaf meal, pork quality improved when the *V. tortilis* leaf meal was included at a rate of 65 and 93 g/kg, respectively.

Dedication

To Those… Who work hard for the Future!

Acknowledgements

I am grateful to my supervisors, Professor M. Chimonyo and Doctor C. Mapiye for their guidance and advisement in the development of this thesis. I would also like to thank Dr B. Ncube and Dr T.J. Zindove for their valuable input to this work. I am grateful to fellow postgraduate students O. Chikwanha (Stellenbosch University), F. Thabethe, V. Hlatini, M. Mchayiya (UFH), S. Ndlela, C. Ncobela, M. V. Mkhwanazi and M. Moyo. A big thank you to my family for their patience while I worked on the study.

This project would not have been a success without the financial support from the University of KwaZulu-Natal.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

List of Appendices

Chapter 1: General Introduction

1.1 Background

In the developing world, meat plays a significant role in reducing malnutrition (Mlambo and Mapiye, 2015), through supply of high quality nutrients (Wyness, 2016). High incidences of obesity, type II diabetes, coronary heart disease (CHD) and cancer has triggered the health awareness of consumers on what they consume (Vahmani *et al*., 2015).

Livestock feeding efficiency is constrained by high dependency on cereal grains such as maize and soya, most of them being grown or imported for human consumption and later feeding livestock. Successful progress has been made in search for alternative non-conventional feed resources in past decades. For example, Vachellia, formerly subgenus *Acacia* (Kyalangalilwa *et al*., 2013), especially *Acacia karroo*, *Acacia nilotica* and *Acacia tortilis* have been assessed in pig feeding (Halimani *et al.,* 2005; Khanyile *et al.,* 2014; Ndou *et al*., 2015, Khanyile *et al*., 2016; Hlatini *et al*., 2016). The maximum inclusion levels of *Vachellia* leaf meals recommended from previous studies were based on pig performance and metabolic response with little or no emphasis on pork quality. It is likely possible that pork quality in general will require different inclusion level to that of growth performance previously recommended. *Vachellia tortilis* may improve pork quality through antioxidant potency and enriched *n*-3 fatty acid. The recently report by Khanyile *et al.* (2016) demonstrated that graded levels of V. tortilis leaf meal reduced back-fat thickness also warrant further research on effect on meat quality in general. Fats play a

vital role in meat quality such as flavor, water holding capacity, colour, tenderness and fatty acid composition. Muscle composition of pigs can be directly influenced by type of diet fed during finishing phase (Dugan *et al*., 2004).

Vachellia leaves contain high level of phenolics and fibre (Dube *et al*., 2001; Halimani *et al.*, 2005). Polyphenolic compounds and high fibre depress feed intake, growth performance and increased size of internal organs (Makkar, 2003; Mueller-Harvey, 2006; Khanyile et al., 2016). Polyphenolic compounds may also have anthelmintic properties (Makkar, 2003) and natural antioxidant (Mapiye *et al.,* 2011) thereby reducing the need of synthetic antioxidants which may predispose humans to various diseases such as cancer, high blood pressure and cardiovascular conditions (Faseseas *et al.*, 2007).

1.2 Justification

The effect of *Vachellia tortilis* leaf meal inclusion on pork quality is poorly understood. Such information is critical in making recommendations of optimum inclusion levels of *Vachellia tortilis* leaf meal in finisher pig diets. The knowledge is critical in addressing concerns on the acceptability of pork product and various diseases including diabetes type II and coronary heart diseases associated with meat consumption. The optimum inclusion levels for maximizing growth performance, pork quality and fatty acid composition are likely to differ (Khanyile *et al.*, 2014). Appropriate inclusion levels of *Vachellia tortilis* may, therefore, depend on whether pig producers wish to sell live pigs to abattoirs at the end of the production cycle or they slaughter and sell pork to consumers.

Where pork is the main interest and other products are to be produced, optimum *Vachellia* inclusion levels should be based on the health indicators of pork, such as *n*-3 fatty acid contents and *n*-6: *n*-3 ratios. *Vachellia trotilis* contains proanthocyanadins which jeopardises feed consumption. Yet, these proanthocyanadins have been reported to act as natural antioxidants that retard lipid oxidation and improve the quality and shelf-life of meat. Utilization of *Vachellia* leaf meal in feeding pigs diet will not only contribute in the reduction of feed costs which accounts for about 70 % of variable costs, but also improve pork quality.

1.3 Objectives

The goal of the study is to examine the use of the abundant but underutilsed Vachellia leaf meal to reduce pressure on soyabean stocks and explore antioxidant activity of leaf meal in pork quality. The broad objective of the study was to assess carcass characteristics, physicochemical properties, fatty acid profile and oxidative stability of pork from pigs fed on graded levels of *Vachellia tortilis* leaf meal-based diet. The specific objectives were to:

1. Assess total phenolic, protein binding capacity and antioxidant activity of *Vachellia* species leaves;
2. Determine the effect of graded levels of *Vachellia tortilis* leaf meal-based diet on growth and carcass characteristics of finishing pigs;
3. Determine the physicochemical properties pork from pigs fed on graded levels of *Vachellia* tortilis leaf meal based diets; and

4. Assess response in fatty acid profile, atherogenecity, thrombogenecity indices and of pork to increasing *Vachellia tortilis* leaf meals.

1.4 Hypothesis

1. *Vachellia tortilis* leaves contains high phenolic compounds, protein binding capacity and antioxidant activity
2. Graded levels of *Vachellia tortilis* leaf meal-based diet may improve carcass characteristics of pigs.
3. *Vachellia tortilis* leaf meal improves physicochemical properties of pork
4. *Vachellia tortilis* leaf meal-based diet has no effect fatty acid, atherogenecity and thrombogenecity indices of pork.

1.5 References

Dube, J.S., Reed J.D., Ndlovu L.R. 2001. Proanthocyanidins and other phenolics in Acacia leaves of Southern Africa. *Animal Feed Science and Technology, 91, 59-67.*

Dugan, M.E.R., Aalus, J.L., Utaro, B. 2004. Nutritional manipulation of pork quality: Current opportunities. *Advances Pork Production, 15, 237-243*

Faseseas, M.K., Mountzouris, C.K., Tarantilis, P.A., Polissiou, M., Zervas, G. 2007. Antioxidant activity in meat treated with oregano and sage essential oils. *Food Chemistry, 106, 1188- 1194*

Halimani, T. E., Ndlovu, L. R., Dzama, K., Chimonyo, M., Miller, B. G., 2005. Metabolic response of pigs supplemented with incremental levels of leguminous *Acacia karroo, Acacia nilotica* and *Colophospermum mopane* leaf meals. *Journal of Animal Science 81, 39 - 45.*

Kayalangalilwa, B., Boatwright, J.S., Daru, B.H., Maurin, O., van der Bank, M. 2013. Phylogenetic position and revised classification of *Acacia s.l.* (Fabaceae: Mimosoideae) in Africa, including new combinations in *Vachellia* and *Senegalia*. *Botanical Journal of the Linnean Society*, 172, 500 -523.

Khanyile, M. Ndou, S.P., Chimonyo, M. 2014. Influence of *Acacia tortilis* leaf meal based diets on growth performance of pigs. *Livestock Science,* 167, 211 – 218.

Khanyile, M., Ndou, S.P., Chimonyo, M. 2016. Influence of *Acacia tortilis* leaf meal-based diet on serum biochemistry, carcass characteristics and internal organs of finishing pigs. *Animal Production Science,* http:// dx. Doi. Org / 10. 1071/ AN 15182.

Hlatini, V.A., Khanyile, M., Zindove, T.J., Chimonyo, M. 2016. Feed intake and growth performance of growing pigs fed on Acacia tortilis treated with Polyethylene glycol. *Tropical Animal Production, 48, 584- 591.*

Makkar, H. P. S., 2003. Effects and fate of tannins in ruminant animals, adaptation to tannins and strategies to overcome detrimental effects of feeding tannins-rich feeds. *Small Ruminant Res*earch, *49, 241- 256*.

Mapiye, C., Chimonyo, M., Dzama, K., Hugo, A., Strydom, P.E., Muchenje, V. 2011. Fatty acid composition of beef from Nguni Steers supplemented with *Acacia karroo* leaf-meal. *Journal of Food Composition and Analysis, 24, 523-528.*

Mlambo, V., Mapiye, C. 2015. Towards household food and nutrition security in semiarid areas: What role for condensed tannin-rich ruminant feedstuffs? *Food Research International, 76, 953-961*.

Mueller-Harvey I. 2006. Review unraveling the conundrum of tannins in animal nutrition and health. *Journal of Science Food and Agriculture, 86, 2010-2037.*

Ndou, S.P., Khanyile, M., Chimonyo, M. 2015. Growth performance and nutrition-related serum metabolites in growing pigs fed on *Acacia tortilis* leaf meal. *Livestock Sci*ence, *182, 22 -27*.

Vahmani, P., Mapiye, C., Prieto, N., Rolland, D.C., McAllister, T.A., Aalhus, J.L., Dugan, M.E.R. 2015. The scope for the manipulating polyunsaturated fatty acid content of beef: A review, *Journal of Animal Science and Biotechnology, 6 (1), 29.*

Wyness, L. 2016. The role of red meat in the diet: Nutrition and health benefits. *Proceedings of the Nutrition Society*, 75, 227-232.

CHAPTER 2: Literature Review

2.1 Introduction

The evaluation of optimum inclusion levels of *Vachellia tortilis* leaf meal in pig diets requires that its impact on pork quality be considered to draw a holistic feeding management strategy. Pork quality can be manipulated through dietary interventions (Nuernberg *et al.*, 2005). There has been a considerable amount of work on the effect of *Vachellia* leaf meal on pig performance and welfare. However, the influence of *Vachellia* leaf meal-based diets on pork quality is poorly understood. This review of literature discusses the antioxidant potential of *Vachellia* leaves, attributes of leaf meal on growth performance of pigs, carcass characteristics, physicochemical properties and fatty acid profiles.

2.2 Nutritional composition of *Vachellia tortilis* leaves

Vachellia is a genus of shrubs and trees which includes about 135 species. Today, most of them are widely spread throughout the arid and semi-arid tropics either as pure stands or in mixtures with allied woody species (Banerji *et al.*, 2007). *Vachellia tortilis*, belonging to family fabaceae (sub family Mimosoideae) is a medium umbrella-shaped tree (Palmer, 1977). The crude protein (CP) content of *Vachellia tortilis* leaves range from 134 to 218 g/kg dry matter (Yadav *et al.,* 2013; Khanyile *et al.,* 2014). The content of neutral detergent fibre (NDF) and acid detergent fibre (ADF) range from 154 to 308 and from 114 to 251 g/kg DM (Dube *et al*., 2001). The utilization of *Vachellia* leaf meal in feeding livestock is limited by its polyphenolic compounds such as proanthocyanidins (Dube *et*

al., 2001). *Vachellia tortilis* leaves contain 51.5 g/kg DM of proanthocyanidins (Khanyile *et al*., 2014). Proanthocyananidins have been reported to act as natural antioxidants in meat (Mapiye *et al*., 2011). Antioxidants are chemical compounds that are capable of donating hydrogen to the free radicals to minimize rancidity and retard lipid peroxidation without damage to the sensory or nutritional properties of meat products (Luhucky *et al.*, 2010).

2.3 Antioxidant potential of *Vachellia* leaf meal

Pork provides high quality protein, vitamins, minerals and fat and contributes significantly in the alleviation of malnutrition in the tropical areas (Qwele *et al*., 2013). The inherent of antioxidants in meat are very low raising serious concerns of safety and shelf life (Kumar *et al.,* 2013). Recently reports on the negative effect of synthetic antioxidant such as butylated hydroxyanisole, butylated hydroxytoluene and tertiary butyl hydroquinone in human heathy (Karakaya *et al.,* 2011), make it imperative to assess the antioxidant potential of non-conventional feed stuffs. This has prompted nutritionists to find alternative natural antioxidants. Various legumes are a rich source of flavonoids besides being good sources of protein, carbohydrates, vitamins and minerals (Kumar *et al*., 2013). Flavonoids are potent natural antioxidants and protect the cells by scavenging and preventing the production and initiation of free radicals, superoxide anions and lipid peroxy radicals (Amarowicz *et al*., 2004). Mapiye *et al*. (2011) reported high antioxidant potency of beef from steers supplemented with *V. karroo*. The information on the antioxidant activity of *V. tortilis* leaves is scarcely documented. Most of work has focused on the quantification of

ant-nutrients such as condensed tannins and total phenolics. The concentrations of total phenolics are given in Table 2.1. Antioxidant activity of the plant extracts can be determined using various assays which includes, the ferric reducing antioxidant power (FRAP) assay, DPPH (2, 2-diphenyl-1-picryhydrazyl), radical scavenging assay and the *β*-carotene-linoleic acid assay.

Table 2.1: Chemical composition and mineral concentration of *Vachellia tortilis* leaves

Parameter	Content (g/kg)
Dry matter	944
Neutral detergent fibre	494
Crude protein	218
Acid detergent fibre	145
Ash	65.0
Condensed tannins	51.5
Ether extract	40.1
Neutral**No table of figures entries found.** detergent insoluble nitrogen	27.4
Acid detergent insoluble nitrogen	18.3
Water holding capacity	6.06
Swelling capacity	4.78
Phosphorus	23.0
Potassium	17.3
Calcium	9.60
Magnesium	3.02
Sodium	0.41
Iron (mg/kg DM)	178
Manganese (mg/kg DM)	2.0135.0
Zinc (mg/kg DM)	19.01
Copper (mg/kg DM)	2.01
Total extractable phenolics	241
Total extractable tannins	226
Total condensed tannins	51.5-77.8
Soluble condensed tannins	18.9

Sources: (Dube *et al.,* 2001; Rubanza *et al.*, 2005; Khanyile *et al*., 2014)

2.4 Fatty composition of *Vachellia* leaf meal

Dietary fatty acids are absorbed directly and unchanged from the intestine of non-ruminant and deposited in the muscle (Enser *et al.,* 2000; Nguyen *et al.,* 2005). It is imperative to assess the fatty acid profile of *V. tortilis* leaf meal. Srivastav and Kumar (2013) reported that, in the *Vachellia tortilis* seed oil, linolenic acid (50.4 %) was the maximum, followed by linoleic acid (36.8 %), Palmitic acid (6.4 %), Oleic acid (4.0 %) and stearic acid (0.6 %). The content of linoleic acid in seed is similar to soya bean, sunflower and vegetable oils having specified co-dex standard range of 48 - 55 %, 48 - 74 % and 68 - 73 %, respectively (Srivastav and Kumar, 2013). Few, if any, information is available on the fatty acid composition of *Vachellia tortilis* leaves.

2.5 Utilization of *Vachellia* leaf meal as an ingredient in pig feeds

Feed deficit is the major constraint to pig production. The fact that pigs have simple stomach like humans make them to compete for grains such as maize and soya beans which are staple food for humans. High feed cost in pig production has resulted in exploitation of non-conventional feed resources such as *Vachellia karroo, V. nilotica* and *V. tortilis* leaf meals (Halimani *et al*., 2005; Khanyile *et al*., 2014). The choice of species largely depends on nutritional composition, anti-nutritional factors and availability. Maximum inclusion levels of *Vachellia* leaf meal in pig's diet has been reported based on growth performance and physiological response of pigs. Few, if any, information is available on influence of *Vachellia* leaf meal of pork quality. It is, therefore, critical that the information on the effect of *Vachellia* leaf meal on pork quality be incorporated when such

recommendations are made. It is likely that optimum inclusion levels for growth performance may differ from those for optimum pork quality.

2.6 Effect of *Vachellia* leaf meal on carcass traits

The use of leaf meal in feeding pigs has been reported by several authors to reduce feed intake, average daily gain which consequently lead to lower slaughter weight (Halimani et al., 2005; Paiva-Martins *et al*., 2009; Khanyile *et al*., 2014). The reduction of growth rate and carcass traits has been always associated with high fibre content of leaf meal and condensed tannins. Inclusion of *V. tortilis* leaf in above 150 g/kg DM of feed in finishing pig diets has been reported to reduce slaughter weight, cold dressed mass, back fat thickness and dressing percentage, respectively (Khanyile *et al.,* 2016). The effect of V. tortilis leaf meal on pH (45 min) and ultimate pH has not been studied. Meat pH is significantly correlated to attributes such as colour and water-holding capacity (Huff-Lonergan et al., 2002), which are crucial traits when consumers make purchasing decision (Brewer and McKeith, 1999). The reduction in back-fat thickness of pigs fed on graded levels of *V. tortilis* leaf meal warrants further investigation on its effects on fatty acid composition of pork. Reduction of fat deposition has been reported to influence fatty acid profile of pork (Dugan *et al*., 2015).

2.7 Physicochemical properties of pork

Reference ranges of physicochemical properties of pork are shown in Table 2.2.

2.7.1 Pork colour

Colour is one of the most important visual characteristics of meat and has major influence on consumer acceptability of the meat product and also purchase decision (Banović *et al*., 2009). The determination of pork colour is done using the commission international De l' Eclairage (CIE) colour system (Commission International De l' Eclairage, 1976). The three fundamental colour coordinates are L^*, a^* and b^*. The L^* measures the lightness (which is a measure of the light reflected (100 – white, 0 =black), a^* is the measure of redness of the meat while b^* measures the yellowness of meat (Commission International De l' Eclairage, 1976). Hue angle, which describes the fundamental colour of a substance, and Chroma, which describes the saturation of a colour, are calculated as $\tan^{-1}(b^*/a^*)$ and $(a^{*2}+ b^{*2})^{0.5}$, respectively (Minolta, 1993).

The colour of pork is influenced by intrinsic factors such as breed, genotype, sex, type of muscle, diet and extrinsic factors such as pre-slaughter handling and slaughter procedure, which significantly decrease pH and ultimate pH (Rosenvold and Andersen, 2003). Ultimate pH is high correlated to meat colour (Huff-Lonergan *et al*., 2002). Generally, high ultimate pH is associated with darker meat colour and decrease in redness (Bidner *et al.,* 2004). Mukumbo *et al*. (2014) reported a decrease in redness in LD muscle from pigs fed on *Moringa* leaf meal, while Mapiye *et al.* (2010) reported an increase in redness (a*) of meat from Nguni cattle supplemented with *V. karroo* leaf meal. The effect of *Vachellia tortilis* leaf meal on pork colour is poorly understood.

Table 2. 1: Ranges of values of some pork quality characteristics as reported in literature

Pork quality characteristics	Range of values	Source
Lightness (L^*)	52 – 58	Van Laack *et al*., 1994
Redness (a^*)	4.00 - 9.82	Lee *et al*., 2012
Yellowness (b^*)	0.73 - 5.52	Lee *et al*., 2012
pH	5.4 – 6.8	Lawrie, 1991; de Vries and Van der Wal, 1992; Lee *et al*., 2012
Ultimate pH	5.40 - 6.52	Lee *et al*., 2012
Cooking loss (%)	20.48 – 44.68	Bertram *et al*., 2003;
Warner Bratzler Shear Force (N)	47.7	De Smet *et al.,* 1998
Moisture (%)	72.7 – 75	Kim, *et al*., 2006
Protein content (%)	21.2 – 24.1	Jacyno *et al*., 2006; Kim *et al*., 2008
Intramuscular fat (%)	1.27 – 3.77	Wood *et al*., 2003

2.7.2 Moisture

Moisture is one of the important quality characteristic in meat and can be measured in several methods which include, drip loss, thaw loss, cooking loss, water binding capacity and total moisture content (Leygonie *et al*., 2012). Moisture loss in meat is influenced by several factors such as decrease in pH, the loss of adenosine triphosphate (ATP) and shrinkage of the myofibrils as a result of rigor mortis (Huff-Lonergan and Lonergan, 2005).

2.7.3 Ultimate pH

Ultimate pH is responsible for 79 % of the variation in colour, 57 % of variation in drip loss, and 77 % of the variation in purge loss (Bidner *et al.,* 2004). Generally, higher pH pork will have superior quality compared to lower pH pork. Yet, pH can have a dramatic effect on shelf-life from both a microbiological and color stability perspective (Holmer *et al.,* 2009). It is generally known that pH affects the freshness, water-holding capacity, and color of muscle (Zhu *et al*., 2011). The pH decline and ultimate pH has a strong influence on pork quality in terms of colour and water holding capacity (Huff-Lonergan *et al*., 2002). Generally, higher pH pork is associated with superior quality compared to lower pH pork (Holmer *et al*., 2009). There are three categories of meat quality, the first one being dark, firm dry (DFD); pale, soft, exudative (PSE) and reddish, firm, non-exudative (RFN). Kim *et al.* (2006) demonstrated a decline in ultimate (pH_{24}) of pork from pigs fed on three levels of fermented permisimmom shell diets (FPSD). The FPSD contain high level of water soluble tannins. The effect of *Vachellia tortilis* leaf meal diet on Ultimate pH in not known.

2.7.4 Intramuscular fat

The intramuscular fat (IMF) content plays a pivot role in the acceptability of pork because it influences quality traits such as flavour, juiciness, cooking loss and shear force of meat (Fisher *et al*., 2000; Thompson, 2002; Joo *et al*., 2013). Feeding strategies is one of the important factors affecting IMF deposition (Dugan *et al*., 2004; Du *et al*., 2010), for example the increase of fibre diet has been reported to decrease IMF of pork (Paiva-Martins *et al*., 2009). In most animals, adipogenesis start at the visceral fat deposit, followed by subcutaneous and intermuscular, lastly intramuscular (Hausman *et al*., 2009). The IMF deposition is positively correlated with general fatness of the body and red muscle fiber but negatively correlated with white muscle type (Hwang *et al*., 2010). The effect of *Vachellia* leaf meal on intramuscular pork has not been studied.

2.7.5 Muscle protein

Postmortem degradation of muscle protein influences the tenderness and cooking loss (Huff Lonergan *et al.,* 2010). Muscle protein degradation is triggered by Low and high ultimate pH (Li *et al.,* 2014). Currently, little information is available on the effect of *Vachellia* leaf on muscle protein of pork.

2.7.6 Tenderness

Tenderness measurements are intended to mimic the forces produced during biting and mastication (Honikol, 1998). Tenderness is the most important eating quality attributes, which influences consumer's perceptions of acceptability of meat

product (Holmer *et al.,* 2009). Factors such as connective tissue, type of muscle fiber, IMF and extent of proteolysis in rigor muscle influence tenderness of meat (Joo *et al.*, 2013). Meat tenderness is measured using a Warner-Bratzler shear force (WBSF). The force in Newton (N) required to shear block of meat perpendicular to its grain of the muscle fiber is determined using an Instron Universal Testing Machine fitted with a Warner-Bratzler shearing device.

2.8 Fatty acid composition of pork

Fatty acids have significantly important role in the nutritive value of meat. Essential unsaturated fatty acids, such as linoleic ($C_{18:2}$), linolenic ($C_{18:3}$) and arachidonic are necessary constituents for mitochondria and cell walls (Wood *et al.,* 2003). Fatty acid composition of pork can be altered through feeding management strategies. Reference values of fatty acid composition of pork from clinical healthy pigs are shown in Table 2.3. Nutritional guidelines recommend reducing fat intake in humans, especially SFA, and minimizing the intake of *n*-6 fatty acids relative to *n*-3 fatty acids (Department of Health, 1994). The imbalance in the *n*-6 to *n*-3 ratio has been associated with numerous diseases, from cardiovascular and inflammatory diseases to diabetes and autoimmune disorders (Dugan *et al*., 2015). The minimum recommended PUFA/SFA ration is 0.4 and the n-6/n-3 ratio is 4:1 (Wood *et al*., 2003). The equations proposed by Ulbricht and Southgate (1991) for the atherogenic and thrombogenic indices indicate that the C12:0, C14:0, and C16:0 FA is atherogenic and that C14:0, C16:0, and C18:0 are thrombogenic. Atherogenicity is defined as formation of atheromatous deposits, especially on the

innermost layer of arterial walls (Véniant *et al.,* 2000). Thrombogenicity refers to the tendency of a material in contact with the blood to produce a thrombus, or clot (Mackman, 2008). The atherogenic index and the thrombogenic indexes are calculated to assess the risk of atherosclerosis (Del Nobile *et al*., 2009; Enser *et al.*, 2000). Atherogenic and thrombogenic indices are calculated using the formulae proposed by Ulbricht and Southgate (1991) as follow:

S: P = (C14:0 + C16:0 + C18:0) /ΣMUFA + ΣPUFA; AI = (C12:0 + 4 × C14:0 + C16:0)/ [ΣMUFA + Σ(n-6) + Σ(n-3)]; TI = (C14:0 + C16:0 + C18:0)/ [0.5 × ΣMUFA + 0.5 × Σ(n-6) + 3 × Σ(n-3) + Σ(n-3)/Σ(n-6)]; where MUFA = monounsaturated FA; PUFA = polyunsaturated FA. Pigs have general higher proportions of the major polyunsaturated fatty acid (PUFA) linoleic acid (18:2n - 6) in both tissues than cattle and sheep (Wood *et al.,* 2008). Saturated and monounsaturated fatty acids in pigs are synthesized *de novo* and, are not easily influenced by diet compared to polyunsaturated fatty acids, linoleic (18:2, n-6) and α-linolenic (18:3, n-3), which cannot be synthesized (Doreau *et al*.,1997; Enser *et al*., 2000; Teye *et al*., 2006). The acceptable firmness of pork fat is achieved when it contains 12 to 15 % linoleic acid and more than 41 % saturated fatty acids (Hugo and Roodt, 2007).

The effect of *Vachellia* leaf meal on fatty acid composition, atherogenic and thrombogenic indices of pork has not been documented. The leaves of *Vachellia* species have high concentration of polyphenols (Dube *et al.,* 2001; Halimani *et al.,* 2007; Khanyile *et al.,* 2014). Polyphenolic compounds have advantages of increasing conjugated linoleic acid concentrations in beef and yield meat of lighter

colour (Mapiye, *et al*., 2011; Patra and Saxena, 2011). Feeding *Vachellia* leave in pigs may directly influence *n*-3 fatty acids in pork since forages are reported as a dietary source of *n*-3 fatty acid to animals (Aurousseau *et al*., 2004). In contrast to ruminants, fatty acids in pigs are not metabolized to any great extent by microbes in the digestive tract prior to lipid digestion and absorption (Doreau *et al*., 1997).

Table 2. 2 Reference values of fatty acid composition of pork from clinical healthy pigs

Fatty acid	Mg/100g
$C_{12:0}$ (lauric)	2.6
$C_{14:0}$ (myristic)	30
$C_{16:0}$ (palmitic)	526
$C_{18:0}$ (stearic)	278
$C_{18:1}$ (trans)	-
$C_{18:1}$ (oleic)	759
$C_{18:2}$ n-6 (linoleic)	302
$C_{18:3}$ n-3 (α-linolenic)	21
$C_{20:3}$ n-6 (lauric)	7
$C_{20:4}$ n-6 (arachidonic)	46
$C_{20:5}$ n-3 (eicosopentaenoic)	6
$C_{22:5}$ n-3 (docosopentaenoic)	13
$C_{22:6}$ n-3 (docosohexaenoic)	8
P:S	0.58
n-6:n-3	7.22

Source: Enser *et al.*, 1996.

2.9 Summary of literature review

Pig production has the potential to contribute positively through supply of protein and income in the tropics. Pig production is limited by shortages of quality feed and competition of ingredients such as grain seeds. Feed shortage is projected to increase with the increase in human population. *Vachellia* leaf meal utilization in feeding pigs is expected to reduce feed cost and improve pork quality. The effects of *Vachellia* leaf meal on pork quality need to be characterized. The broad objective of the current study was, therefore, to determine the effect of *Vachellia tortilis* leaf meal on the physico-chemical properties and fatty acid profile of pigs.

2.10 References

Amarowicz, R., Pegg, R.B., Rahimi-Moghaddam, Barl, B., Weil, J.A. 2004. Free-radical scavenging capacity and antioxidant activity of selected plant species from the Canadian prairies. *Food chemistry, 84, 551-562*.

Aurousseau, B., Bauchart, D., Calichon, E., Micol, D., Priolo, A. 2004. Effect of grass or concentrate feeding systems and rate of growth on triglyceride and phospholipid and their fatty acids in the M. Longissimus thoracis lambs. *Meat Science, 66, 531-541*.

Banerji, R., Shukla, P., Bajpai, A., B. S. Dixit, B.S. 2007. Review Article: Omega-3 Fatty Acid. *Applied Botany Abstracts*, *27, 315-333*.

Bertram, H.C, Andersen, H.J., Karlsson, A.H., Horn, P., Hedegaard, J., Lars Nørgaard, L., Engelsen, S.B. 2003. Prediction of technological quality (cooking loss and Napole Yield) of pork based on fresh meat characteristics. *Meat Science, 65, 707-712.*

Bidner, B. S., Ellis, M., Brewer, M. S., Campion, D., Wilson, E. R., McKeith, F. K. 2004. Effect of ultimate pH on the quality characteristics of pork. *Journal of Muscle Foods*, 15(2), 139–154.

Brewer, M. S., McKeith, F. K. 1999. Consumer-rated quality characteristics as related to purchase intent of fresh pork. *Journal of Food Science, 64(1), 171-174.*

CIE, (1976). Colorimetry, 2nd ed. Commission International de l'Eclairage, Vienna, Switzerland.

de Vries, A.G. van der Wal, P.G. 1992. Breeding for quality. In: E Puolane and D.I. Demeyer, (Eds).

Department of Health, 1994. Nutritional aspects of cardiovascular disease. Report on Health and Social Subjects No. 46, London, UK.

Del Nobile, M.A., Conte, A., Incoronato, A.L., Panza, O., Sevi, A., Marino, R. 2009. New strategies for reducing pork back-fat content in typical Italian Salami. *Meat Science, 81, 263-269.*

De Smet, S., Casteels, M., Van de Voorde, G., Spincemaille, G., Barekmans, D. 1998. Meat and carcass quality in two pig lines of different stress susceptibility genotype and crosses. *Animal Science*, *66, 441-447*.

Doreau, M., Chilliard, Y. 1997. Digestion and metabolism of dietary fat in farm animals. *British Journal of Nutrition*, *78, S15–S35.*

Du, M., Yin, J., Zhu, M. J. 2010. Cellular signaling pathways regulating the initial stage of adipogenesis and marbling of skeletal muscle. *Meat Science*, *86, 103–109.*

Dube, J.S., Reed, J., Ndlovu L.R. 2001. Proanthocyanidins and other phenolics in Acacia leaves of Southern Africa. *Animal Feed Science and Technol*ogy, *91, 59-67.*

Dugan, M.E.R., Vahmani, P., Turner, T.D., Mapiye, C., Juårez, M., Prieto, Beaulieu, A.D., Zijlstra, Patience, J.F. Aalhus, J.L. 2015. Pork as a source of Omega-3 (n-3) fatty acids. *Journal of Clinical Medicine*, *4, 1999-2011*.

Enser, M.S., Hallet, K., Hewitt, B., Fursey, G.A.J., Wood, J.D. 1996. Fatty acid content and composition of English beef, lamb and pork retail. *Meat Science*, *42, 443-456.*

Enser, M., Richardson, R.I., Wood, J.D., Gill, B.P., P. R., Sheard, P.R. 2000. Feeding linseed to increase the n-3 PUFA of pork: fatty acid composition of muscle, adipose tissue, liver and sausages. *Meat Science, 55, 201-212.*

Fisher, A.V., Enser, M., Richardson, R.I., Wood, J.D., Nute, G.R., Kurt, E. 2000. Fatty acid composition and eating quality of lamb types derived from four diverse breed production systems. *Meat Science*, 55, 141 -147.

Halimani, T. E., Ndlovu, L. R., Dzama, K., Chimonyo, M., Miller, B. G., 2005. Metabolic response of pigs supplemented with incremental levels of leguminous *Acacia karroo, Acacia nilotica* and *Colophospermum mopane* leaf meals. *Animal Science, 81, 39 – 45.*

Halimani, T.E., Ndlovu, L.R., Dzama, K., Chimonyo, M., Miller, B.G., 2007. Growth performance of pigs fed on diets containing *Acacia karroo, Acacia nilotica* and *Colophosperamum mopane* leaf meals. *Tropical Animal Health and Production*, *40, 125-137.*

Hausman, G. J., Dodson, M. V., Ajuwon, K., Azain, M., Barnes, K. M., Guan, L. L., Jiang, Z., Poulos, S. P., Sainz, R. D., Smith, S., Spurlock, M., Novakofski, J., Fernyhough, M. E., Bergen, W. G. 2009. Board-invited review: The

biology and regulation of preadipocyte and adipocytes in meat animals. *Journal of Animal Science, 87, 1218-1246*.

Holmer, S.F., McKeith, R.O., Bole, D.D., Dilger A.C., Eggert, J.M., Petry, D.B., McKeith, F.K., Jones, K.L., Killefer J. 2009. The effect of pH on shelf-life of pork during aging and simulated retail display. *Meat Science, 82, 86-93.*

Huff-Lonergan, E., Baas, T. J., Malek, M., Dekkers, J. C., Prusa, K., Rothschild, M. F. 2002. Correlations among selected pork quality traits. *Journal of Animal Science, 80, 617–627.*

Huff-Lonergan, E., Lonergan, S.M. 2005. Mechanisms of water holding capacity of meat: the role of post mortem biochemical and structural changes. *Meat Science, 71, 194-204*.

Huff Lonergan, E., Zhang, W., Lonergan, S.M. 2010. Biochemistry of postmortem muscle: Lessons on mechanisms of meat tenderization," *Meat Science*, *86, 184-195.*

Hugo, A., E. Roodt. 2007. Significance of porcine fat quality in meat technology: A review. *Food Reviews International, 23, 175-198*.

Hwang, Y. H., Kim, G. D., Jeong, J. Y., Hur, S. J., Joo, S. T. 2010.The relationship between muscle fiber characteristics and meat quality traits of highly marbled Hanwoo (Korean native cattle) steers. *Meat Science*, 86, *456-461*.

Jacyno, E., Pietruszka, A., Kołodziej, A. 2006. Influence of pig meatiness on pork meat quality. *Polish Journal of Food Nutrition Science 15/56 (2), 137-140.*

Joo, S.T. Kim, G.D., Whang, YH., Ryu, Y.C. 2013. Control of fresh meat quality through manipulation of muscle fiber characteristics. A review. *Meat Science*, *95, 828-836*.

Karakaya, M., Bayrak, M., Ulusoy, K. 2011. Use of natural antioxidant in meat and meat products. *Journal of Food Science and Engineering, 1, 1 -10.*

Kim, H.Y., Song, Y.M., Jin, S., Kim, II-S., Kang, Y., Lee, S., Chopwappa, R., Ha, J., Kang, S. 2006. Effect of change in meat quality parameters on pig *Longissimus dorsi* muscle by the addition of fermented persimmom shell diet. *Asian-Australian Journal of Animal Science, 19, 286 – 291*.

Kim, J.H., Seong, P.N., Cho, S.H., Park, B.Y., Hah, K.H., Yu, L.H., Lim, D.G., Hwang, I.H., Kim, D.H., Lee, J.M., Ahn, C.N., 2008. Characterization of nutritional value for twenty-one pork muscles. *Asian-Australian Journal of Animal Science, 21, 138-143.*

Khanyile, M., Ndou, S.P., Chimonyo, M. 2014. Influence of Acacia tortilis leaf-based diets on growth performance of pigs. *Livestock Science*, *167, 211-218.*

Khanyile, M., Ndou, S.P., Chimonyo, M. 2016. Influence of *Acacia tortilis* leaf meal-based diet on serum biochemistry, carcass characteristics and internal organs of finishing pigs. *Animal Production Sci*ence, http:// dx. Doi. Org / 10. 1071/ AN 15182.

Kim, H., Song, Y., Jin, S., Kim, S., Kang, Y., Lee, S., Chowdappa, R., Ha, J., Kang, S. 2006. The effect of change in meat quality parameters on pig

Longissimus dorsi muscle by the addition of fermented persimmon shell diet. *Asian-Australian Journal of Animal Science, 2, 286-291.*

Kumar, P., Kumar, S., Tripathi, M.K., Mehta, N., Ranjan, R., Bhat, Z.F., Singh, P.K. 2013. Flavonoids in the development of functional meat products: A review, *Vet World, 8, 573-578*.

Lawrie, R. A. 1991. Meat Science. In Pergamon Press. Cambidge, England.

Lee, K.H., Jung, S., Kim, H.J., Kim II.S., Lee, J.H., Jo, C. 2012. Effect of dietary supplementation of the combination of gallic and linoleic acid in thigh meat of broilers. *Asian-Australian Journal of Animal Science, 25, 1641-1648*.

Leygonie, C., Britz, T.J., Hoffman, L.C. 2012. Impact of freezing and thawing on the quality of meat: Review. *Meat Science, 91, 93-98.*

Li, P., Wang, T., Mao, Y., Zhang, Y., Niu, R.L., Zhu, L., Luo, X. 2014. Effect of Ultimate pH on postmortem myofibrillar protein degradation and quality characteristics of Chinese yellow crossbreed cattle. *Scientific world Journal*, http://dx.doi.org/10.1155/2014/174253.

Luhucky, R., Nuernberg, K., Kovac, L., Bucko, O., Nuermberg, G. 2010. Assessment of the antioxidant potential of selected plant extracts-In vitro and in vivo experiments on pork. *Meat Science, 85, 779-784.*

Mackman, N. 2008. Trigers, targets and treatment for thrombosis. *National Institute of Heath, 451 (7181), 914-918.*

Mapiye, C., Chimonyo, M., Dzama, K., Strydom, P.E., Muchenje, V. 2010. Meat quality attributes of Nguni steers supplemented with *Acacia karroo* leaf-meal. *Meat Science*, *8, 621-627*.

Mapiye, C., Chimonyo, M., Marufu, M. C., Dzama, K., 2011. Utility of *Acacia karroo* for beef production in Southern Africa smallholder farming systems: A review. *Animal Feed Science and Technology, 164, 135-146.*

Paiva-Martins, F.S, Barbosa, S., Pinheiro, V., Mourão, J.L., Monteiro, D.O. 2009. The effect of olive leaves supplementation on the feed digestibility, growth performances of pigs and quality of pork meat. *Meat Science, 82, 438- 443*.

Minolta, (1993). Precise colour communication. Minolta Camera, Ltd., Ramsey, New Jersey.

Mukumbo, F.E., Maphosa, V., Hogo, A., Nkukwane, T.T., Mabusela, T.P., Muchenje, V. 2014. Effect of Moringa Oleifera leaf meal on finisher pig growth performance, meat quality, shelf life and fatty acid composition of pork. *South African Journal of Animal Science*, *44, 388-400.*

Nguyen, L. Q., Nuijens, M.C.G.A., Everts, H., Salden, N., Beynen. A.C. 2005. Mathematical relationships between the intake of *n*-6 and *n*-3 polyunsaturated fatty acids and their contents in adipose tissue of growing pigs. *Meat Science, 65, 1399-1406.*

Nuernberg, K., Fischer, K., Nuernberg, G., Kuechenmeister, U., Klosowska, D., Eliminowska-Wenda, G. 2005. Effects of dietary olive and linseed oil on lipid composition, meat quality, sensory characteristics and muscle structure in pigs. *Meat Science, 70, 63–74.*

Palmer, E., 1977. A field guide to the trees of Southern Africa. William Collins Sons & Co LTD, London.

Patra, A. K., Saxena, J. 2011. Exploitation of dietary tannins to improve rumen metabolism and ruminant nutrition. *Journal of the Science of Food and Agriculture*, *164(3), 135–146*.

Rosenvold, K., Andersen, H.J. 2003. Factors of significance for pork quality- a review, *Meat Science, 64*, 219-237.

Rubanza, C.D.K., Shem, M.N., Otsyina, R., Bakengesa, S.S., Ichinohe, T., Fujihara, T. 2005. Polyphenolics and tannins effect on in vitro digestibility of selected *Acacia* species leaves. *Animal Feed Science and Technology, 119, 129–142.*

Srivastava, M., Kumar, S. 2013. Fatty acid compositional studies of lesser known *Acacia tortilis* seed oil. *Food and Nutritional Sciences, 4, 59-62*.

Teye, G. A., Sheard, P. R., Whittington, F. M., Nute, G. R., Stewart, A., Wood, J. D. 2006. Influence of dietary oils and protein level on pork quality. 1. Effects on muscle fatty acid composition, carcass, meat and eating quality. *Meat Science, 73, 157–165.*

Thompson, J. 2002. Managing meat tenderness. *Meat Science, 62, 295-308*.

Ulbricht, T.L.V., Southgate, D.A.T. 1991. Coronary heart disease seven dietary factors. *Lancet, 338, 985-992.*

Van Laack, R.L.J.M., Kauffman, R.G., Sybesma, W., Smulders, F.J.M., Eikelenboom, G., Pinheiro, J.C. 1994. Is colour brightness (L-value) a reliable indicator of water holding capacity in porcine muscle? *Meat Science, 38, 193-201.*

Véniant, M.M., Sullivan, M.A., Kim, S.K., Ambroziak, P Alice Chu, A., Martha D. Wilson, M.D., Hellerstein, M.K., Rudel, L.L. Rosemary L. Walzem, R.L., Young, S.G. 2000. Defining the atherogenicity of large and small lipoproteins containing a lipoprotein B100. *The Journal of Clinical investigation, 106 (12), 1501-1510.*

Wood, J.D. Richardson, R.I., Nute, G.R., Fisher, A.V., Campo, M.M., Kasapidou, E., Sheard, P.R., Enser, M. 2003. Effects of fatty acids on meat quality: A review. *Meat Sci*ence, *66, 21-32.*

Wood, J.D., Nute, G.R., Richardson, R.I., Whittington F.M., Southwood, O., Plastow, G., Mansbridge, R., da Costa, N., Chang, KC. 2004. Effects of breed, diet and muscle on fat deposition and eating quality in pigs. *Meat Science*, *67, 651-667*.

Wood, J.D., Enser, M., Fisher, A.V., Nute, G.R., Sheard, P.R., Richardson, R.I., Hughes, S.I., Whittington, F.M. 2008. Fat deposition, fatty acid composition and meat quality: A review. *Meat Science, 78, 343 -358.*

Yadav, P., Kant, R., Kothiyal, P. 2013. A review on *Acacia tortilis*. *International Journal o*f *Pharmaceutical and Phytophamacological Research*, *3 (2), 93-96.*

Zhu, X., Ruusunen, M., Gusella, M., Zhou, G., Puolanne, E. 2011. High post-mortem temperature combined with rapid glycolysis induces phosphorylase denaturation and produces pale and exudative characteristics in broiler Pectoralis major muscles. *Meat Science, 89(2), 181-188.*

Chapter 3: Protein-precipitating capacity and antioxidant activity of leaf extracts from five selected *Vachellia species*

Abstract

Mathanolic extracts from the leaves of *Vachellia xanthophloe, V. robusta, V. tortilis, V. nilotica* and *V. nigrescens* were compared for their protein precipitating capacity and antioxidant activities. *Vachellia tortilis* leaves exhibited high phenolic content with a correspondingly high protein precipitating capacity. Total phenolics ranged from 1.7×10^{-8} mg/mg to 5.89×10^{-7} mg/mg. *Vachellia tortilis* and *V. xanthophloe* exhibited high protein precipitating capacity (54.2 and 52.0 %, respectively). The lowest protein precipitating capacity was recorded for *V. nilotica* (9.2 %). The concentration of an antioxidant which induces a response halfway between the baseline and maximum after a specified exposure time (EC_{50}) values of the extracts in the 2, 2-diphenyl-1-picryhydrazyl (DPPH) free radical scavenging assay ranged from 0.570 µg/ml to 0.762 µg/ml, compared to 0.481 µg/ml for ascorbic acid. A dose-depended curvilinear response was obtained for *V. xanthophloe, V. robusta, V. tortilis* and *V. nigrescens* in the ferric-reducing power assay. All extracts, except *V. tortilis* exhibited high antioxidant activity (81.6 to 90.7 %) comparable to butylated hydroxytoluene (BTH) (94.8 %) based on the rate of *β*-carotene bleaching. The oxidation rate ratio (ORR) values of these extracts (0.09 to 0.18) were also comparable to that of BTH (0.08). Results of the study showed that *Vachellia* leaf meals have high antioxidant potency which may improve colour stability and retard lipid oxidation in meat.

(Under Review in *Food Chemistry*)

Keywords: *β*-carotene-linoleic acid; extracts; Ferric Reducing Antioxidant Power; Pronthocynadins; 2, 2-diphenyl-1-picryhydrazyl

3.1 Introduction

The presence of polyphenolic compounds in the leaf meal has always been viewed as the limiting factor hindering the potential utilization of *Vachellia* leaf meal in feeding livestock (Dube *et al.,* 2001; Hlatini *et al.*, 2016). Phenolic compounds such as proanthocyanidins are capable of binding proteins, thereby making them unavailable for digestion and absorption. Poor feed efficiency and low body weight gain in pigs and chickens fed on *Vachellia* leaf meal has been ascribed to the adverse effects of polyphenolics. Proanthocyanidins can precipitate protein but there is very little understanding as to the extent to which each species can precipitate protein. Information on the protein precipitating capacity assists feed compounders and nutritionists during diet formulation. If meat quality and safety is a concern than growth, moderate intake of phenols can enhance meat quality thereby serving as natural antioxidants (Mapiye *et al.,* 2011).

Antioxidants play a major role in prevention of lipid oxidation in meat products (Shahidi and Wanasundara, 1992). Synthetic antioxidants such as *tert*-butylhydroxytoluene, *tert*-butylhydroxyanisole, and *tert*-butylhydroquinone are widely used to reduce or delay lipid oxidation in foods (Amarowicz *et al*., 2004). In the past two decades, there has been growing concern over the use of synthetic

antioxidants due to their toxicological concerns (Rababah *et al.,* 2004; Moyo *et al.,* 2010) and this has discouraged the use of synthetic antioxidants (Hayes *et al*., 2010) and prompted researchers to explore potent natural antioxidants (Mapiye *et al.,* 2011).

One area where natural anti-oxidants have a huge potential for use is in meat production. Incorporating plants that contain antioxidants in livestock diets has the potential to retard lipid oxidation, microbial growth (Djenane *et al*., 2003) and improve colour stability (Zinoviadou *et al.,* 2009), thus, increasing the shelf life of meat. It increases the quality and shelf life of meat and thus accepted by consumers at the point of purchase (Arnold *et al*., 1992). *Vachellia* leaf meal has been widely used in feeding livestock in the tropics due to feed shortages and in reducing costs. *Vachellia* leaf meal is reported to have high levels of phenolic compounds (Abdulrazak *et al*., 2000; Dube *et al.,* 2001; Khanyile *et al.,* 2014), which possesses natural antioxidant (Mapiye *et al*., 2011,). *Vachellia* species are commonly used for fencing and as a fire wood (Mapiye *et al.,* 2010). They are also used for medicinal purposes (Ali *et al*., 2012). For example, *V. nilotica* has been used for treatment of cancer, cold, cough, diarrhoea, dysentery, fever, gall bladder infections, haemorrhoid, ophthalmia, sclerosis, small pox, tuberculosis, leprosy, bleeding piles, leucoderma and menstrual abnormalities (Gowri *et al*., 2011).

The choice of species to use in livestock feed largely depend on protein composition and proanthocyanidins content and more often the species with high

protein and low proanthocyanidins content is most preferred, often overlooking the antioxidant potency of these species. The antioxidation benefits from the leaf meal could be passed to meat and meat product to enhance quality. Few reports could be found on protein binding capacity and antioxidant potential of *Vachellia xanthophloe, V. robusta, V. tortilis, V. nilotica* and *V. nigrescens* leaves. The objective of the current study was, therefore, to compare the protein binding capacity and antioxidant potential of *Vachellia xanthophloe, V. robusta, V. tortilis, V. nilotica* and *V. nigrescens* leaves. It was hypothesized that phenolic composition, protein precipitating capacity and antioxidant activity varies with the species.

3.2 Materials and methods

3.2.1 Chemicals

Bovine serum albumin, sodium dodecyl sulphate (SDS), SDS-triethanolamine solution, methanol, 7% (v/v) triethanolamine, ferric chloride reagent, gallic acid, 2, 2-diphenyl-1-picryhydrazyl (DPPH), ascorbic acid, butylated hydroxyethylene (BHT) were obtained from BDH chemicals Ltd. (Poole England). Potassium phosphate, potassium ferricyanide, trichloroacetic acid, *β*-carotene, chloroform, linoleic acid and Tween 20 were obtained from BDH chemicals Ltd. (Poole England). All chemicals used in these assays were of an analytical grade.

3.2.2 Study site and harvesting of leaves

Eight trees of each *Vachellia* species leaves (*V. tortilis, V. robusta, V. nilotica, V. nigrescens* and *V. xanthophloea*) were harvested at Makhathini Research Station, Jozini, KwaZulu-Natal Province, South Africa, in the post- rainy season (April, 2016). The study site has a latitude of S27º 23´ 42 45 and a longitude E32º 10´ 48 48. The mean annual rainfall for the area is between 588 and 635 mm. The maximum temperature is 32 ºC while minimum temperature is 10ºC. Freshly collected leaves were washed thoroughly under a running tap of water, and dried at room temperature (25 ºC) for 4 days under shade. Leaves were separately ground into fine powders through a 1mm sieve screen and stored in sealed polyethylene bags at ambient conditions until further analyses.

3.2.3 Sample extraction

Five grams of powdered *Vachellia* leaf samples from each of the eight trees of the same species were pooled as unique samples and extracted using 50 mL of 50 % aqueous methanol (v/v) in a sonication bath for 1 hour. These extracts were filtered under vacuum using Whitman's No. 1 filter paper and concentrated under vacuum using a rotary evaporator at 35 ºC before being completely dried under a stream of cold air.

3.2.4 Determination of protein-precipitating capacity of *Vachellia* leaves

The determination of the protein-precipitating capacity of the phenolics in the 50 % aqueous methanol extracts was determined according to Makkar (1999).

Briefly, to 2 ml of bovine serum albumin (BSA) solution (1 mg BSA/ml in acetate), increasing concentrations of 50 % aqueous methanol extracts (0.95, 0.90, 0.85, 0.80, 0.75, 0.70 ml) were added and the total volume made up to 3 ml with 50 % aqueous methanol in triplicates in centrifuge tubes. These contents were then vortexed and kept at 4 ºC overnight in a refrigerated centrifuge. After 16 h of incubation, tubes were centrifuged at 3000 *g* for about 10 min and the supernatant carefully removed without disturbing the precipitate. To the supernatant, 1.5 ml of 1 % (w/v) sodium dodecyl sulphate (SDS) solution was added and vortexed until dissolved completely.

Aliquots (1 ml) of dissolved phenolic-protein complex were transferred into clean sets of test tubes in which 3 ml of SDS-triethanolamine solution [1 % SDS (w/v) and 7 % (v/v) triethanolamine in distilled water] were added, followed by 1 ml ferric chloride reagent (0.01 M ferric chloride in 0.1 M HCL). Absorbance readings were taken at 510 nm using a UV- visible spectrophotometer after 30 min incubation period at room temperature. Obtained readings were converted to gallic acid equivalents, using a gallic acid as the standard curve.

Different aliquots of the extracts were made up to 1 ml with 1 % of SDS, and 3ml of the SDS- triethanolamine solution were added, followed by 1m of ferric chloride

reagent. After incubation at room temperature for 30 min, the absorbance at 510 nm was obtained as described above. A linear regression curve between phenolic acid equivalents and mg sample (in the aliquot taken) was drawn using Graph Pad Prism version 6 software. The percentage of total phenolics which can precipitate protein was calculated using the formula:

(x/y) x100).

X and y are variables describing the ratio betweenphenolics precipitating and the weight of plant sample of the curve (mg phenolics precipitated/mg plant samples) representing the protein precipitating and total phenolics in the sample respectively.

3.2.5 Determination of antioxidant activity of *Vachellia* leaves

Antioxidant activities were assessed using three models. The radical scavenging, ferric reducing antioxidant power (FRAP) assay and the β-carotene-linoleic acid assays were used.

3.2.5.1 Radical scavenging assay

Free radical scavenging activity was measured using the method described by Karioti *et al*. (2004). Fifteen microliters of each plant extract was diluted using methanol (735 µl) and then added to a methanolic DPPH solution (750 µl, 0.1 mM) to give a final volume of 1.5 ml in the reaction mixture. The concentration of 2, 2-diphenyl-1-picryhydrazyl (DPPH) in the final reaction was 50 µM. The DPPH

solution was prepared freshly before the assay. The stock solution of leaf extract consisted of a range of concentrations of (0.065, 0.26, 0.52, 1.04, 6.25, 12.5 and 50 mg/ml, giving final reaction mixture concentrations of 0.65, 2.6, 5.2, 10.4, 65, 125, 250 and 500 µg/ml, respectively). The reaction mixtures were prepared under dim light and incubated at room temperature for 30 min in the dim light. Decrease in the purple colouration of the reaction mixtures was measured using a spectrophotometer at 517 nm. The absorbance measurements were done in the dim light. A standard antioxidant, ascorbic acid (5, 10, 20, 40, 80 µm was used as a positive control. A solution with the same chemicals except for the extracts or standard antioxidants was used as negative control. Methanol was used to blank the spectrophotometer. The absorbance reading of the extracts without DPPH were recorded and subtracted from the corresponding readings with DPPH (background correction). Each test extract was done in triplicate. The free radical scavenging activity (RSA) as determined by the decolouration of the DPPH solution was calculated according to the formula,

% RSA= $(1- A_E/ A_D) \times 100$

Where A_E is the absorbance of the reaction mixture containing the standard antioxidant or extract, and A_D is the absorbance of the DPPH solution only. Radical scavenging activity (%) was plotted against the extract concentration

3.2.5.2 Ferric reducing antioxidant power (FRAP) assay

The ferric-reducing powers of the *Vachellia* species leaf extracts were determined using the method described by Lim *et al.* (2009) with slight modifications. A 30 µl

volume of each plant extract, ascorbic acid or butylated hydroxytoluene (0.26 mg/ml) dissolved in methanol was added to a 96-well micro-plate and two-fold serially diluted. Subsequently, potassium phosphate buffer (40 µl, 0.2 M, pH 7.2) and potassium ferricyanide (40 µl, 1% w/v) were added. The reaction mixtures were incubated at 50 ºC for 20 min. Following the incubation period, trichloroacetic acid (40 µl, 10 % w/v), distilled water (150 µl) and $FeCl_3$ (30 µl, 0.1 % w/v) were added, before incubation at room temperature for 30 min in the dark. Absorbance was measured at 630 nm using an ELISA plate reader (Jonfia-Essien *et al.,* 2008). The ferric-reducing power capacity of the extracts and standard antioxidants were plotted on graph of absorbance against concentration. Samples for the assay were prepared in triplicate.

3.2.5.3 β-carotene-linoleic acid assay

The coupled inhibition of *β*-carotene and linoleic acid oxidation was measured according to the method described by Amarowicz *et al*. (2004) with modifications. Dried *Vachellia* leaves extracts and BHT (positive control) were re-dissolved in 50 % aqueous methanol to a concentration of 6.25 mg/ml. *β*-carotene (10 mg) was dissolved in 10 ml chloroform in a brown Schott bottle. Excess chloroform was evaporated under vacuum, leaving a thin film of *β*-carotene. Linoleic acid (200 µl) and Tween 20 (2 ml) were immediately added to the *β*-carotene. Aerated distilled water (497.8 ml) was added to the mixture, to give a final *β*-carotene concentration of 20 µg/ml. The mixture was further saturated with oxygen by vigorous agitation to form an orange-coloured emulsion. The emulsion (4.8 ml) was dispensed into

test tubes to which *Vachellia* leaves extracts or BHT (200 µl, 5 mg/ml) were added, giving a final concentration of 250 µg/ml in the assay. Initial absorbance at 470 nm (t = 0) for each reaction mixture was measured immediately after adding the sample extract or BHT. Absorbance values were then obtained every 30 min for 2 h, with incubation in a water bath at 50 ºC. A Tween 20 solution was used as a blank. The negative control consisted of 50 % aqueous methanol without sample extract. The rate of *β* -carotene bleaching was calculated using the following formula:

Rate of *β*-carotene bleaching = In ($A_{t=0}$ / $A_{t=t}$) x1/t

Where $A_{t=0\ is}$ the absorbance of the emulsion at 0 min; and $A_{t=t}$ is the absorbance at time, t (30, 60, 90 min). The average rate of *β* -carotene bleaching was then calculated based on rates at 30, 60 and 90 min. The calculated average rates were used to determine the antioxidant activity (ANT) of the sample extracts, and expressed as percentage inhibition of the rate of *β* -carotene bleaching using the formula:

% ANT = ($R_{control} - R_{sample}$) / R_{sample} x100

Where $R_{control}$ and R_{sample} represent the respective average *β* -carotene bleaching rates for the negative control and plant extracts. Antioxidant activity was further expressed as the oxidation rate ratio (ORR) based on the equation:

ORR = R_{sample} /$R_{control}$

Antioxidant activity (AA) was calculated as described by Braca *et al*. (2003), based on the inhibition of coupled oxidation of b-carotene and linoleic acid against the negative control at t = 60 min and t = 120 min using the formula:

$\% AA = [1- (A_0 – A_t)]/ (A_{00} – A_{0t}) \times 100$

Where, A_0 is the absorbance of the test extract

A_t, absorbance of test extracts at *t* = 60 and 120 min

A_{00}, absorbance of negative control at *t* = 0

A_{0t}, absorbance of negative control at *t* = 60 and 120 min, respectively.

3.2.6 Statistical analyses

Data on total phenolic, protein precipitating activity and antioxidant capacity of the *Vachellia* leaf extracts were analysed using the General Linear Model (GLM) procedure of SAS (2008) that accounted for the effect of species. Pair-wise comparison of means was performed using the PDIFF option (SAS, 2008). Differences among means were considered significant when $P < 0.05$. A linear regression curve between phenolics precipitated as gallic acid equivalents and mg dry plant samples (in aliquot taken for the assay) was plotted using Graph Pad Prism V6 (Graph Pad Prism® software incl. La Jolla, CA, USA). The slope of the curve (mg phenolics precipitated/mg leaf samples = x) represented the protein-precipitating phenolics in each sample (Makkar, 1999). The EC_{50} values, representing the amount of extract required to decrease the absorbance of DPPH by 50% were calculated from the logarithmic non-linear regression curve derived from the plot data using the PDIFF procedure $P < 0.05$ (SAS, 2008).

3.3 Results

3.3.1 Protein precipitating activity

Total phenolic and protein precipitating activity of *V. xanthophloe, V. robusta, V. tortilis, V. nilotica* and *V. nigrescens* leaf extracts are shown in Table 3.1. There were significant differences (P <0.05) in total phenolics among leaves of *Vachellia* species. *Vachellia Xanthophloe* and *V. nigrescens* had high concentration of total phenolics, while the lowest concentration was recorded for *V. nilotica*. Interestingly, the protein precipitating phenolics showed a similar trend to that of total phenolics. The highest concentration of protein precipitating phenolics was recorded for *V. xanthophloe* and *V. nigrescens*. Lowest concentration was noted for *V. nilotica.*

There were significant differences (P < 0.05) in protein precipitating activity among leaves of *Vachellia* species. Protein precipitating capacity ranged from 9 to 54 %. *Vachellia tortilis* and *V. xanthophloe* had the highest (P < 0.05) percentage of protein precipitating capacity while the lowest (P< 0.05) percentage was recorded for *V. nilotica*

3.3.2 Ferric Ion reducing antioxidant power

Figure 1 depicts the ferric reducing power of *V. xanthophloe, V. robusta, V. tortilis, V. nilotica* and *V. nigrescens* leaf extracts. The reducing power of the *Vachellia*

leaf extracts increased with increasing concentration of these extracts. *Vachellia nigrescens* demonstrated significantly ($P < 0.05$) higher reducing power, while least reducing power was recorded from *V. nilotica.* The reducing power of *V. nigrescens*, *V. xanthophloea* and *V. robusta* was consistently high ($P < 0.05$) to that of BHT at all concentrations tested. *Vachellia tortilis* also showed high ($P < 0.05$) reducing power over BHT in lower concentration, however, beyond 0.4 mg/ml, BHT showed higher ($P < 0.05$) reducing power to that of *V. tortilis*. *Vachellia nilotica* exhibited lower reducing power at all concentrations compared to other leaf extracts.

Table 3.1: Total phenolic and protein precipitating activity of *Vachellia* leaf extracts

Species	PPP (x) ≠	TP (y)*	PPC (%)
V. xanthophloe	$2.16 \times 10^{-3} \pm 1.22 \times 10^{4c}$	$4.18 \times 10^{-3} \pm 4.11 \times 10^{-4b}$	52.0 ± 2.20^{d}
V. robusta	$9.39 \times 10^{-4} \pm 1.1 \times 10^{-5b}$	$3 \times 10^{-3} \pm 2.11 \times 10^{-4a}$	31.32 ± 1.30^{b}
V. tortilis	$9.19 \times 10^{-4} \pm 4.9 \times 10^{-5b}$	$1.7 \times 10^{-3} \pm 8.1 \times 10^{-5a}$	54.2 ± 0.32^{d}
V. nilotica	$8.8 \times 10^{-5} \pm 4 \times 10^{-6a}$	$9.61 \times 10^{-4} \pm 1 \times 10^{-6a}$	9.2 ± 0.43^{a}
V. nigrescens	$2.12 \times 10^{-3} \pm 1.2 \times 10^{-5c}$	$5.89 \times 10^{-3} \pm 2.38 \times 10^{-4b}$	36.1 ± 1.66^{c}

PPP: Protein-precipitating phenolics (mg phenolics precipitated/ mg leaf extract); TP (mg phenolics precipitated/ mg leaf extract): Total phenolics (mg phenolics precipitated/ mg leaf extract); PPC: Protein precipitating capacity; ^{abc} Different letters in the same column represent significantly different values ($P < 0.05$).

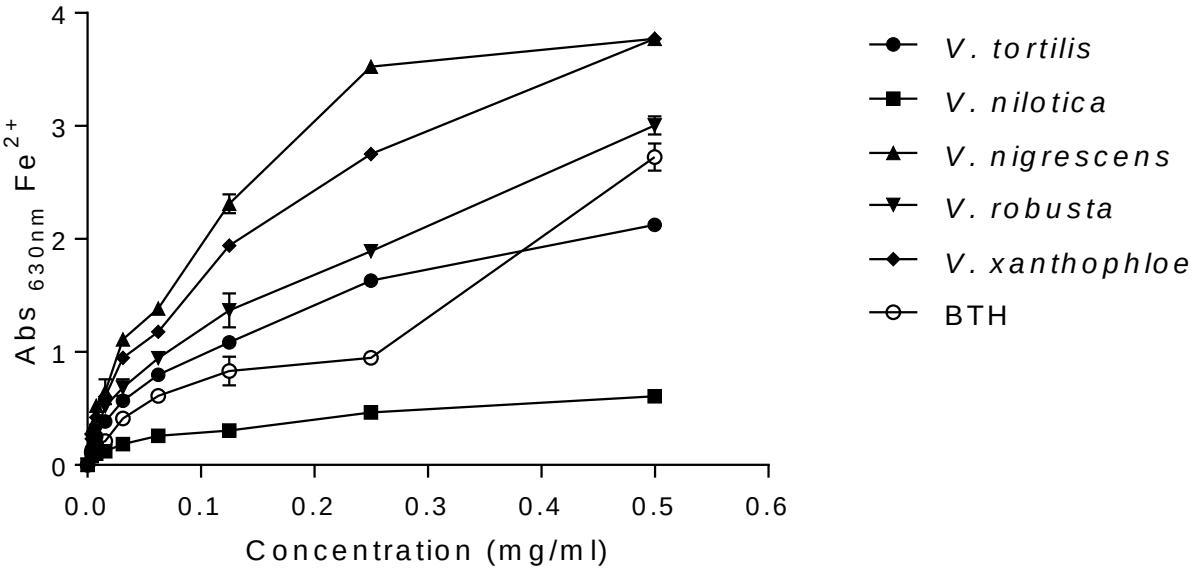

Figure 3.1: Antioxidant activity of *Vachellia* leaves extracts as assessed by ferric ion reducing antioxidant power

3.3.3 *β*-carotene-linoleic acid

The antioxidant activity calculated based on the average rate of *β*-carotene bleaching ranged from 72.3 to 90.7 % (Table 3.2). The highest potency in the assay was recorded for *V. robusta*, while the lowest potency was recorded for *V. tortilis*. For the oxidation rate ratio (ORR), all extracts except *V. tortilis* were not significantly different ($P > 0.05$) to that of the control (BHT). The ORR of the extracts ranged from 0.09 ± 0.04 to 0.28 ± 0.00. The highest ORR was recorded for *V. robusta* while the lowest ORR was recorded for *V. tortilis*. Lower oxidation rate ratio (ORR) values, like EC_{50} values, denote better antioxidant potential. Based on ORR, the order of antioxidant capacity with respect to the protection of *β*-carotene against bleaching was as follows; *V. robusta* > *V. nigrescens* > *V. xanthophloea* > *V. nilotica* > *V. tortilis* ($P< 0.05$).

3.4.4 Radical scavenging activity

The concentration of *Vachellia* leaves extracts required to decrease DPPH by 50 % is shown in Figure 3.2. There was a significant difference ($P < 0.05$) in DPPH radical scavenging activity among the *Vachellia* species. The DPPH radical scavenging activity of *Vachellia* leaf extracts ranged from 0.570 to 0.762 µg/ml. *Vachellia tortilis* demonstrated high ($P < 0.05$) scavenging activity, while *Vachellia robusta* exhibited the lowest ($P < 0.05$) scavenging activity. Radical scavenging activities of *V. xanthophloe*, *V. tortilis* and *V. nigrescens* leaf extracts are comparable ($P > 0.05$) to that of ascorbic acid. Radical scavenging activities of *V. robusta* and *V. nilotica* were significantly lower ($P <0.05$) to that of ascorbic acid.

Table 3.2: Antioxidant activity of *Vachellia xanthophloe, V. robusta, V. tortilis, V. nilotica* and *V. nigrescens as assessed by the coupled oxidation of β*-carotene-linoleic acid

Vachellia	*β*-carotene-linoleic acid	
	ANT (%)	ORR
V. xanthophloe	87.94 ± 4.80^{bc}	0.14 ± 0.04^{ab}
V. robusta	90.70 ± 4.03^{c}	0.09 ± 0.04^{a}
V. tortilis	72.25 ± 0.37^{a}	0.28 ± 0.00^{c}
V. nilotica	81.57 ± 3.47^{b}	0.18 ± 0.03^{b}
V. nigrescens	87.36 ± 0.65^{bc}	0.13 ± 0.01^{ab}
BTH	94.83 ± 3.29^{bc}	0.08 ± 0.03^{ab}

ANT (%) = antioxidant capacity; BHT= butylated hydroxytoluene ORR= oxidation rate ratio; ^{abc} Different letters in the same column represent significantly different values ($P < 0.05$) as separated by PDIFF option (SAS, 2008)

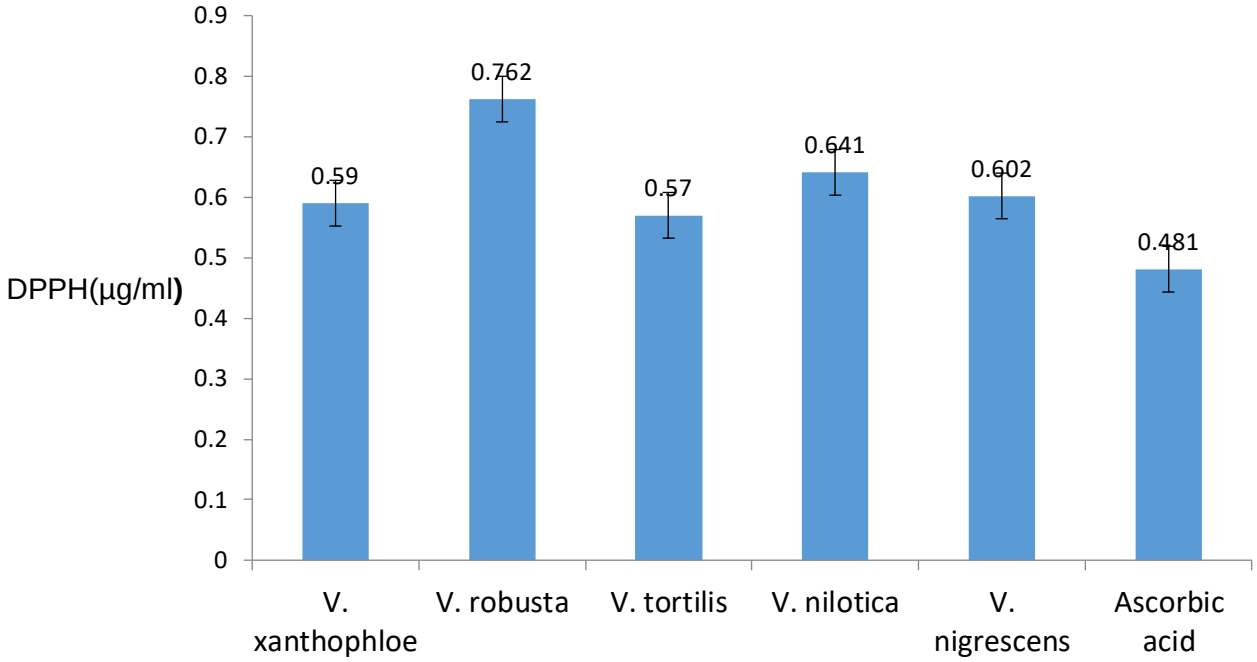

Figure 3.2: Scavenging effect of *Vachellia* leaf extracts on 2, 2-diphenyl-1-picryhydrazyl (DPPH)

3.4 Discussion

Although *Vachellia* leaf meal has been reported for its higher nutritious composition and as a possible substitute for grains seed in pig's diets, little if any, scientific information is available on protein precipitating capacity and antioxidant activity of this leaf meal. The differences in protein-precipitating phenolics concentration among *Vachellia* extracts could be ascribed to the differences in both quality and quantity phenolic compounds present in each species. Classes of phenolic compounds present in plant leaves includes among others flavonoids, hydrolysable tannins and proanthocyanidins often referred to condensed tannins (CT). Proanthocyanidins form complexes with plant proteins thereby making them unavailable for digestion and absorption in livestock (Makkar, 2003). Khanyile *et al*. (2014) reported differences in concentrations of proanthocyanidins in similar *Vachellia* leaves species tested in the current study. It has been reported that pigs produce salivary proline rich protein to cope with tanniferous diet, however, damages in liver and mucosal lining in the small intestines has been reported when the leaf meal exceed 130 g/kg DM (Halimani *et al.*, 2005; Khanyile *et al.,* 2016).

Differences in total phenolic composition in *Vachellia* species in the current study could be as a result of genetic differences among species. A similar observation was also reported in the results of Dube *et al*. (2001) and Abdulrazak *et al*. (2000) who reported different concentrations of total phenolics in different *Vachellia* species. The total phenolic content of the leaves is affected by plant species, genotype, environment and stage of plant growth (Foley *et al*., 1999). Despite the

negative effect of phenolics in animal nutrition, recently there has been growing interest on the natural antioxidant effect of phenolic compounds. Phenolic compounds have been reported to improve colour stability and retard lipid oxidation in meat (Mapiye *et al*., 2011; Qwele *et al.,* 2014) and it is reported that proanthocyanidins have a high anti-oxidant activity compared to other phenolic compounds (Krishnaiah, *et al.,* 2009).

The differences in protein precipitating capacity of *Vachellia* leaf meal in the current study could be due to the differences in total phenolic composition. Interestingly, *V. tortilis* exhibited low total phenolic content but high protein precipitating capacity, a phenomenon that points to the quality (class composition of phenolics) as being one of the major determinant factors in the protein binding capability of extracts. Although different phenolic compounds were not characterized in this study, based on the results obtained, it can be speculated that protein precipitating capacity could have been influenced by type of phenolic compounds. The protein precipitating capacity of *Vachellia* extracts obtained in this study provide an important basis and decision guiding principle on the consideration of the inclusion levels in pig diets. Recently, studies have revealed that *Vachellia* leaf meal should range between 100 – 130g/kg DM in growing pig diets (Halimani *et al.*, 2005; Khanyile *et al*., 2014).

Vachellia leaf extracts were assessed for their ability to reduce the F^{3+}/ ferricyanide complex to the ferrous (F^{2+}) form. In this assay, yellow colour of the test solution

changes to various shades of green and blue depending upon the reducing power of each extract. The current study showed that *Vachellia* leaves extracts were able to reduce the F^{3+}/ ferricyanide to ferrous (F^{2+}) form. The differences in ferric-reducing power observed among extracts could be ascribed to the differences in total phenolic concentration observed in *Vachellia* leaf extracts. For example, *V. xanthophloe, V. robusta* and *V. nigrescens* had high phenolic concentration, consequently exhibiting high capacity to act as electron donors, indicating their potential to react with free radicals, thus increasing the antioxidant potency (Amarowicz *et al.,* 2004). Moyo, Ndhlala *et al*. (2010) also indicated a direct relationship between reducing power effects of plant extracts and the content of phenolic compounds. High concentration of phenolic compounds exhibited by *Vachellia* extracts in this study suggest that they are potential sources of antioxidant (Jonfia-Essien *et al.,* 2008).

The *β*-carotene-linoleic acid assay measures the ability of a plant extract to prevent or minimize the coupled oxidation of *β* -carotene and linoleic acid in an emulsified aqueous system (Pajero *et al*., 2002). In this model, *β* –carotene undergoes rapid discolouration in the absence of an antioxidant. During oxidation, an atom of hydrogen is abstracted from the active bis-allylic methylene group of linoleic acid located on carbon-11 between two double bonds (Frankel, 1998). The pentadienyl free radical so formed then attacks highly unsaturated *β* –carotene molecules in an effort to reacquire a hydrogen atom. As the *β* –carotene molecules lose their conjugation; the carotenoids lose their characteristic orange colour.

Presence of a phenolic antioxidant can prevent the extent of *β* –carotene destruction by neutralizing the linoleate free radical and any other free radicals formed within the system (Amarowicz *et al.,* 2004). Based on the phenolic concentration of extracts in the current study, by direct translation, one would have expected that *V. robusta, V*. *xanthophloe* and *V. nigrescens* exhibit high percentage of antioxidant and lower ORR. Interestingly, however, *V. nilotica* had lower concentration of total phenols in this study but exhibited comparable percentage of antioxidant and ORR value to those of *V. robusta*, *V. xanthophloe* and *V. nigrescens*. Different phenolic compounds show different colorimetric responses and that the molecular antioxidant response of phenolic compounds to a lipid substrate varies remarkably depending on chemical structure and oxidation conditions (Amarowicz and Shahidi, 1995). Despite these differences in antioxidant percentages and ORR values among the *Vachellia* extracts, results of the current study show that *Vachellia* leaf meal can be exploited as natural antioxidant in pork.

The effect of antioxidants on DPPH is due to their hydrogen donating ability (Karioti *et al.,* 2004). The purple colour of the freshly prepared DPPH solution fades when an antioxidant compound is present in the test solution to quench DPPH. The antioxidant molecule provides a hydrogen atom or donates an electron to DPPH (Amarowicz *et al*., 2004). The fact that DPPH radical scavenging abilities of *V. tortilis, V. xanthophloe* and *V. nigrescens* extracts were comparable to that of

ascorbic acid positions these extracts as potential antioxidant in pg diets. Phenolic compounds originating from plant extracts possess antioxidant activity (Amarowicz *et al*. 2009). Pajero *et al*. (2002) also demonstrated high correlation (0.91 to 0.99) between the total phenolic content and three method of scavenging activity. *Vachellia* leaves extracts showed a propensity in quenching DPPH free radicals. Radical scavenging activity of extracts is also associated with lipid content in leaves (Iqbal *et al*., 2012). Species with high lipid content possess high levels of ascorbic acid (Iqbal *et al*., 2012). Khanyile *et al*. (2014) reported that *Vachellia tortilis* contained high levels of lipids (40.1g/kg DM) to those of the similar species being tested in the current study.

In all three assays, species that exhibited high phenolic compounds and high protein precipitating capacity demonstrated high antioxidant potency. For example, *V. Xanthophloe, V. robusta, V. nigrescens and V. tortilis* extracts had high total phenolic and high protein precipitating capacity which consequently increases their antioxidant potency. Generally, the present study has demonstrated potent antioxidant activity of *Vachellia* leaf meal and thus may be exploited as an important source of improving the natural antioxidant status of meat from pigs.

3.5 Conclusions

Vachellia xanthophloe leaf extracts has high antioxidant activity. All other extracts were comparable to that of synthetic antioxidant. The choice of species to be used will also be influenced by the availability. *Vachellia* leaf meal could be explored

and exploited as a natural source of antioxidant in meat, consequently improving colour stability and retarding lipid oxidation. It is worth assessing the relationship between graded levels of *V. tortilis* leaf meal and growth performance and carcass characteristics and meat quality in general a sthe the continuation from previous work documented else where by Khanyile et al., 2014; Khanyile et al., 2016.

3.7 References

Abdulrazak, S.A., Orden, E. A., Ichinohe, T., Fujhara, T. 2000. Chemical composition, phenolic concentration and in vitro gas production characteristics of selected *Acacia* fruits and leaves. *Journal of Animal Science, 7, 935-940*.

Ali, A., Akhtar, N., Khan, B.A., Khan, M.S., Rasul, A., UZ-Zaman, S., Khalid, N., Waseem, K., Mahmood, T., Ali, L. 2012. *Acacia nilotica*: a plant of multipurpose medicinal uses. *Journal of Medical Plants Research*, 6, 1492-1496.

Amarowicz, R., Shahidi, F. 1995. Antioxidant activity of green tea catechins in a *β* –carotene-linoleate model system. *Journal of Food Lipids, 2, 47-56*.

Amarowicz, R., Pegg, R.B., Rahimi-Moghaddam, Barl, B., Weil, J.A. 2004. Free-radical scavenging capacity and antioxidant activity of selected plant species from the Canadian prairies. *Food Chemistry, 84, 551-562*.

Amarowicz, R., Estrella, I., Hernández, T., Dueñas, M., Traszynska, A., Kosinska, A., Pegg, R.B. 2009. Antioxidant activity of a Red Lentil extract and its fractions. International *Journal of Molecule Science*, *10, 5513-5527.*

Arnold, R. N., Scheller, K. K., Arp, S. C., Williams, S. N., Buege, D. R., Schaefer, D. M. 1992. Effect of long- and short-term feeding of alpha-tocopheryl acetate to Holstein and crossbred beef steers on performance, carcass characteristic, and beef color stability. *Journal of Animal Sci*ence, *70, 3055-3065.*

Djenane, D., A. Sánchez-Escalante, J.A. Beltrán, P. Roncalés. 2003. Extension of the shelf life of beef steaks packaged in a modified atmosphere by treatment with rosemary and displayed under UV-free lighting. *Meat Science*, *64, 417-426*.

Dube, J.S., Reed J.D, Ndlovu, L.R. 2001. Proanthocyanidins and other phenolics in *Acacia* leaves of Southern Africa. *Animal Feed Science Technol*ogy, *91, 59-67*.

Frankel, E.N. 1998. Hydroperoxide formation. In *Lipid oxidation* (*pp. 23-41*). Dundee: The Oily Press.

Foley, W.J., Iason, G.R., McArthur, C. 1999. Role of plant secondary metabolites in the nutritional ecology of mammalian herbivores: how far have we come in 25 years? In: Jung, H-J.G. and Fahey Jr., G.C. ed. Nutritional Ecology of herbivores. Proceedings of the fifth international symposium on the nutrition of herbivores, April 11-17, 1999 in San Antonio, Texas USA.130-209, American Society of Animal Science, Savoy, Illinois USA.

Gowri, S.S., Pavitha, S., Vasantha, K. 2011. Free radical scavenging capacity and antioxidant activity of young and barks of *Acacia nilotica* (L.) DEL.

International Journal of Pharmacy and Pharmaceutical Science, *1, 160-164.*

Halimani, T. E., Ndlovu, L. R., Dzama, K., Chimonyo, M., Miller, B. G. 2005. Metabolic response of pigs supplemented with incremental levels of leguminous *Acacia karroo, Acacia nilotica* and *Colophospermum mopane* leaf meals. *Animal Science, 81, 39- 45.*

Hayes, J. E., Allen, P., Brunton, N., O'Grady, M.N., Kerry, J. P. 2010. Phenolic composition and in-vitro antioxidant capacity of four commercial phytochemical products: Olive leaf extract (Olea europium L), lutein, sesamol and ellagic acid. *Food Chemistry, 126, 948-955.*

Hlatini, V.A., Khanyile, M., Zindove, T.J., Chimonyo, M. 2016. Feed intake and growth performance of growing pigs fed on Acacia tortilis treated with Polyethylene glycol. *Tropical Animal Production 48, 584- 591.*

Iqbal, S., Younas, U., Sirajuddin, Chan, K.W., Safra, R.A., Uddin, M.K. 2012. Proximate composition and antioxidant potential of leaves from three varieties of Mulberry (*Morus* sp.): a comparative study. *International Journal of molecule Science 13, 6651-6664*.

Jonfia-Essien, W.A., West, G., Alderson, P.G., Tucker, G. 2008. Phenolic content and antioxidant capacity of hybrid variety cocoa beans. *Food chemistry 108, 1155-1159.*

Karioti A., Hadjipavlou-Latina, D., Mensah, M.L.K., Fleischer, T.C., Saltsa, H. 2004. Composition and antioxidant activity of the essential oils of *Xylopia aethiopica* (Dun) A. Rich. (Annonaceae) leaves stem bark, root back, and

fresh and dried fruits, growing in Ghana. *Journal of Food Chemistry, 52, 8094-8098.*

Khanyile, M. Ndou, S.P., Chimonyo, M. 2014. Influence of *Vachellia tortilis* leaf meal based diets on growth performance of pigs. *Livestock Science,* 167, *211-218*.

Khanyile, M., Ndou, S.P., Chimonyo, M. 2016. Influence of Acacia tortilis leaf meal-based diet on serum biochemistry, carcass characteristics and internal organs of finishing pigs. *Animal Production Science http:// dx. Doi. Org / 10. 1071/ AN 15182.*

Krishnaiah, D., Devi, T., Bono, A., Sarbatly, R. 2009. Studies on phytochemical constituents of six Malaysian medicinal plants. *Journal of Medical Plant Research 3(2), 67-72.*

Lim, T. Y., Lim, Y. Y., Yule, C. M. 2009. Evaluation of antioxidant, antibacterial and anti-tyrosinase activities of four Macaranga species. *Food Chemistry, 114, 594.*

Makkar, H. P. S. 1999. Quantification of tannins in tree foliage. A laboratory manual for the FAO/IAEA coordinated research project on 'Use of nuclear and related techniques to develop simple tannin assay for predicting and improving the safety and efficiency of feeding ruminants on the tanniniferous tree foliage' (*pp*. *1-29*). Vienna, Austria: Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture.

Makkar, H. P. S. 2003. Effects and fate of tannins in ruminant animals, adaptation to tannins and strategies to overcome detrimental effects of feeding tannins-rich feeds. *Small Ruminant Research, 49, 241-256.*

Mapiye, C., Chimonyo, M., Dzama, K., Strydom, P.E., Muchenje, V. 2010. Meat quality attributes of Nguni steers supplemented with *Acacia karroo* leaf-meal. *Meat Science*, *8, 621-627*.

Mapiye, C., Chimonyo, M., Dzama, K., Hugo, A., Strydom, P. E., Muchenje, V. 2011. Fatty acid composition of beef from Nguni steers supplemented with Acacia karroo leaf-meal. *Journal of Food Composition and Analysis*, *24, 523–528*.

Moyo, M., Ndhlala, A.R., Finnie, J.F. Van Staden, J. 2010. Phenolic composition, antioxidant and acetylcholinesterase inhibitory activities of *Sclerocarya birea* and *Hypephyllum Caffrum* (*Anacardiaceae*) extracts. *Food Chemistry, 123, 69-76.*

Qwele, K., Hugo, A., Oyedemi, S.O., Moyo, B., Masika, P.J., Muchenje, V. 2013. Chemical composition, fatty acid content and antioxidant potential of meat from goats supplemented with Moringa (Moringa oleifera) leaves, sunflower cake and grass hay. *Meat Science, 93, 455-462.*

Pajero, I., Viladomat, F., Bastida, I., Rosas-Romero, A., Flerlage, N., Burillo, I. 2002. Comparison between the radical scavenging activity and antioxidant activity of six distilled and non-distilled Mediterranean herbs and aromatic plants. *Journal of Agriculture and Food Chemistry, 50, 6882-6890.*

Rababah, T.M., Hettiarachchy, N.S., Horax, X. 2004. Total phenolics and antioxidant activities of fenugreek, green tea, grape seed, ginger, rosemary, gotu kola, and ginkgo extracts, vitamin E, and *tert*-butylhydroquinone. *Journal of Agriculture and Food Chemistry, 52, 5183-5186.*

SAS, 2008. SAS User's Guide: Statistics, Version9.1. SAS Institute, Cary, NC, USA

Shahidi, F., Wanasundara, P. K. 1992. Phenolic antioxidants. *Critical Review Food Science and Nutrition, 32*, *67-103*.

Zinoviadou, K.G., Koutsoumanis, K.P. Biliaderis, C.G. 2009. Physico-chemical properties of whey protein isolate films containing oregano oil and their antimicrobial action against spoilage flora of fresh beef. *Meat Science, 82, 338-345.*

Chapter 4: Effect of graded levels of *Vachellia tortilis* leaf meal inclusion on growth performance and carcass characteristics of finishing pigs

(Under Review in *Tropical Animal Health and Production*)

Abstract

The objective of the current study was to determine the effect of *Vachellia tortilis* leaf meal based diet on growth performance and carcass characteristics of 48 Large White x Landrace male pigs (8 pigs/treatment), averaging 63 ± 0.93 (SD) kg BW), randomly allotted to six diets containing 0, 30, 60, 90, 120 and 150 g/kg DM of *V. tortilis* leaf meal, respectively. Average feed intake (ADFI), average daily gain (ADG) and gain: feed (G: F) ratio were recorded weekly. Pigs were slaughtered at 18 weeks of ageat a commercial abattoir and their carcass characteristics were assessed. Overall, ADFI increased linearly (Y= 0.061025x + 2.155240; $P < 0.05$) with inclusion of *V. tortilis.* There was a linear decrease ($P < 0.05$) in ADG and G: F ratio with increase in *V. tortilis* inclusion. The ADG and G: F decreased linearly (Y = -0.006291x + 0.925080; Y= -0.006299 + 0.414923; $P < 0.05$) with inclusion level of *V. tortilis* leaf meal. The inclusion of *V. tortilis* linearly ($P < 0.05$) decreased slaughter weight, warm carcass weight, cold carcass weight, cooler shrink and back-fat thickness. Dressing percentage, pH at 45 minutes (pH_{45}) and temperature at 45 minutes ($temperature_{45}$) were not ($P > 0.05$) affected by *V. tortilis* inclusion. Increasing the inclusion of *V. tortilis* was accompanied by an increase in the negative effect in ADG, G: F, slaughter weight, warm carcass weight, cold weight and back-fat thickness.

(Under Review in *Tropical Animal Health and Production*)

Keywords: Average feed intake; Back-fat thickness; cooler shrink; diets; dressing percentage

4.1 Introduction

In the tropics, pig production contributes significantly in food security and household income (Chimonyo *et al.,* 2005). High feed cost and unavailability of conventional protein supplements are, however, one of the main constraints to efficient pig production (Martens *et. al*., 2012). It is generally known that feed cost constitutes 70% in pig production while maize and soybean meal are the main staples in the diet for pigs. In the past decades, progress has been made in an attempt to reduce the proportion of maize and soybeans in feeding pigs. Utilization of local available non-conventional feed resources, such as *Vachellia* leaf meals, should be considered (Halimani *et al*., 2007; Khanyile *et al*., 2014; Ndou *et al*., 2015).

Vachellia species are the most common bush encroaching species in the communal areas of Southern Africa (Mapiye *et al.*, 2009). *Vachellia tortilis* leaves contain up to 220 g/kg DM of crude protein (CP) and adequate minerals (Dube *et al*., 2001; Khanyile *et al*., 2014). Despite its nutritious composition, *V. tortilis* leaves contain high level of fibre and polyphenolic compounds (Abdulrazak *et al*., 2000). High fibre and polyphenolic compound levels reduce digestibility of proteins,

carbohydrates and lipids (Hansen *et al.*, 2006). Polyphenolic compounds such as proanthocyanidins (condensed tannins) bind with nutrients, thereby reducing their availability (Makkar, 2003). Proanthocyanidins act as natural antioxidants and improve meat colour (Mapiye *et al*., 2010).

Feeding *Vachellia karroo*, *Vachellia nilotica* above 100g/ kg and *Vachellia tortilis* leaf meal above 130 g/kg has been reported to reduce feed intake, average daily gain and feed efficiency (Halimani *et. al*., 2005; Khanyile *et al*., 2014; Ndou *et al*., 2016). To date recommendations on the maximum inclusion levels of *Vachellia* leaf meal in pig diets has been based on growth and healthy aspects of pigs with little emphasis on carcass characteristics, pork quality and fatty acid profile. The previous studies on the effect of *Vachellia* leaf meal ignored the influence of lower levels on carcass characteristics and pork quality. The study was re-designed using lower inclusion levels ranging from 30 to 150g /kg DM to investigate growth performance, carcass characteristics and pork quality in general. The augment is that lower levels such as 30 and 60 might not significant reduce feed cost but greatly improve quality of pork, consequently increasing consumer acceptance of pork. Therefore, the objective of the current study was to determine the effect of feeding graded levels of *V. tortilis* leaf meal on growth and carcass characteristics of pigs. It was hypothesized that *Vachellia tortilis* leaf meal could improve growth and carcass characteristics in pigs.

4.2 Material and methods

The protocol for the experiment was reviewed and approved by the University of KwaZulu-Natal ethics committee (AREC/101/015D).

4.2.1 Study site

The trial was conducted at Ukulinga Research Farm, UKZN, Pietermaritzburg, South Africa. The farm is located in a subtropical hinterland at 29º24'E and 30º24'S with altitude of 775 m. The climate is characterized by mean annual maximum and minimum temperatures of 25.7 and 8.9ºC. During the rainy season, there is a mean annual rainfall of 735 mm, whilst light to moderate frost occurs in the cool dry season.

4.2.2 Diets

Vachellia tortilis leaves were harvested at Makhathini Research Station, Jozini, KwaZulu-Natal Province, South Africa, according to the method described by Khanyile *et al.* (2014). Diets were formulated using Winfeed Feed Formulation Software to contain similar levels of crude protein and energy (140g/kg DM and 17MJ/kg). *Vachellia tortilis* leaf meal was included at a rate of 0, 30, 60, 90, 120 and 150 kg DM, respectively. All experimental diets were supplemented with vitamins and minerals to meet the National Research Council (NRC, 2012) recommended specification for finishing pigs. Diets were not supplemented with any antibiotics or growth promoters. Ingredient compositions of experimental diets are shown in Table 4.1.

4.2.3 Pigs and housing

Forty-eight male Large White pigs with a mean body weight of 63.6 ± 0.73 kg (mean ± SD) aged 14 weeks were used in a completely randomized design. Each pig, representing an experimental unit, were housed in an individual pan with slatted floor under control environment and randomly allotted to each of the six diets for 32 days of experimental period. Pigs were allowed 10 days of adaptation period to the experimental diets. Each pen was fitted with a pre-weighed bin feeder (Big Dutchman Lean Machine©, Postfach). Diets were offered *adlibitum* to pigs and they had free access to clean water, which was provided through low pressure nipple drinkers fitted opposite to the bin feeder.

4.2.4 Chemical analyses of experimental diets

Samples of experimental diets were oven dried at 60 ºC for 4 days, and finely milled through a 1 mm screen in Thomas Wiley Mill Modell 4 (Thomas Scientific; Swedesboro, NJ), and thoroughly mixed before analyses. Diets were analyzed for dry matter (DM), lipids, crude protein (CP), GE, ash and minerals as described by AOAC (2002; method. 942.05). Lipid content of feed was measured by ether extraction after acid hydrolysis with HCl using an XT15 fat extractor and HCl hydrolysis system (ANKOM Technology, Macedon, NY) according to the method described by Lee *et al*. (1996). Fatty acid content of the *V. tortilis* leaves and diets were determined according to the method described by Folch *et al*. (1957). Neutral detergent fibre (NDF) was analyzed according to Robertson and Van Soest (1977), with addition of sodium sulfite and alpha amylase. Acid detergent fibre (ADF) was analyzed according to the method described by Goering and Van Soest (1970).

Natural detergent fiber and ADF values were expressed without residual ash. Condensed tannins were estimated by colorimetrically using the butanol-HCL method (Reed *et al.,* 1982). Bulk density (BD) of the experimental diets was determined according to the water displacement method described by Peterson and Baumgardt (1971). The density of the diet was expressed in g/ml. Water holding capacity (WHC, g water/g dry feed) of these feeds was measured using modifications of the two methods of centrifugation and filtration described by Robertson and Eastwood (1981).

4.2.5 Growth performance

Average daily feed intake (ADFI) was determined by weighing the amount feed offered to individual pig and amount of feed left over in the next morning. Pigs were weighed weekly to determine growth rate of and average daily gain (ADG) for each animal. Feed efficiency which is gain: feed ratio (G: F) for each pig was calculated by dividing ADG by ADFI.

Table 4.1: Ingredient composition of experimental diets (g/kg DM)

Ingredient (g/kg)	Diets					
	0	30	60	90	120	150
Maize	458	432	406	382	357	333
Wheat bran	356	346	337	326	315	304
Soybean 46	86	91.2	94.4	101	106	111
V. tortilis leaves	0	30	60	90	120	150
Oil-sunflower	60.1	61.1	62.0	62.9	63.8	64.8
Limestone	20.9	20.4	19.9	19.5	18.9	18.2
Monocalcium phosphate	9.61	9.9	10.2	10.5	10.8	11.1
Salt	4.9	5.0	5.02	5.03	5.03	5.05
≠Vitamin-mineral premix	1.5	1.5	1.5	1.5	1.5	1.5
$_{L}$-Lysine-HCL	1.64	1.44	1.24	1.05	0.85	0.65
Threonine	0.97	0.86	0.75	0.64	0.52	0.4
Methionine	0.38	0.42	0.46	0.5	0.54	0.58

≠Provides (/kg of DM of diet): vitamin A, 4.8 mg; vitamin D_3, 0.09 mg; vitamin E, 50 mg; vitamin K_3 (43%), 1.0 mg; vitamin B_1, 1.6 mg; vitamin B_2, 2.6 mg; niacin (99.5%), 33.6 mg; vitamin B_{12} , 0.01 mg; vitamin B_6 98%, 2.0 mg; choline (chloride 60%), 121 mg; folic acid (96% pure), 0.48 mg; biotin, 0.18 mg; calcium pantothenate (98%), 5.2 mg; zinc balitracin, 90.0 mg; manganese sulphate, 120.0 mg; zinc, 100 mg; copper, 8 mg; potassium iodide (Iodine 76.45 %), 0.4 mg; cobalt sulphate, 0.2 mg; ferrous sulphate, 100.0 mg and selenium, 0.32 mg on dolomite carrier.

4.2.6 Carcass characteristics

At the end of the experimental period of 32 days, all pigs weighing 86 ± 1.03 (Mean ± SD) were transported to Ambleside abattoir in Winterton, South Africa, KwaZulu-Natal Province, and 135 km from the experimental station. Each pig was weighed to determine slaughter weight (SW). Pigs were slaughtered after 24 hours of fasting period. Only clean water was provided *ad libitum*. They were electrically stunned (300 V for 3 s) with a pair of stunning tongs, shackled by the right leg and exsanguinated while hanging. After removal of hairs, carcasses were eviscerated and split down the center of the vertebral column before being suspended in the air and chilled at 4 °C (1 m/s; 90 % relative humidity) for 2 hours. Carcasses were weighed to determine the hot weight and cold weight (CW) 24 hours post mortem. The dressing percentage (DP) was determined by expressing the HW as a percentage of SW. Cooler shrink (CS), which is the amount of water lost from a carcass in the first 24 to 48 hours after harvest, was calculated using the following formula:

(1 – (cold carcass weight / hot carcass weight) x100.

4.2.7 Determination of pH, temperature and backfat thickness

Post mortem pH, and temperature at 45 minutes ($pH_{45}$and $temperature_{45}$) were measured using portable pH meter equipped with a spear tip electrode and an automatic thermometer compensator probe (CRISON pH25, CRISON instrument SA, Spain). Before pH measurement, the instrument was calibrated with pH 7.0 ± 0.02 and 4.0 ± 0.02 buffers. The pH meter electrode was rinsed using distilled

water between each measurement to avoid treatment contamination. Back-fat thickness was measured using an ultrasound sonography probe at the P2 site as described by Gardner *et. al.* (1990).

4.2.8 Statistical analysis

Statistical analyses for growth performance and carcass characteristics were performed using Proc Mixed procedures of Statistical Analysis Systems Institute (SAS, 2008). The model incorporated dietary *V. tortilis* inclusion levels as the main effect. The pig was the experimental unit and its initial body weight was used as a covariate. The model used was:

$Y_{ij} = \mu + D_i + E_{ij}$

Yij = response variables (growth performance and carcass characteristics)

μ = overall mean

Di = effect of diet (i = 0, 30, 60, 90, 120, 150)

E_{ij} = residual error.

The regression model (PROC REG) procedure of SAS (2008) was used to determine the relationships between each of performance parameters with inclusion level of *V. tortilis* leaf meal during each week of successive feeding. Differences between least-square means were compared using the PDIFF option of SAS (2008). The significance threshold was set at $P < 0.05$.

4.3 Results

Table 4.2 shows the chemical composition of experimental diets. The total CP content ranged from 138 to 148 g/kg, respectively, which meets the protein level specified for growing-finishing pigs (NRC, 2012). The level of starch in the diet decreased significantly ($P < 0.05$) with inclusion level of *V. tortilis* leaf meal. The ADF also showed a decreasing ($P < 0.05$) trend with increase inclusion level of *V. tortilis* leaf meal; however, NDF increase significantly ($P< 0.05$) with inclusion levels of *V. tortilis* leaf meal. The decreae in ADF could be related to the decrease of wheat bran. Wheat bran is the major source of ADF (Lindberg, 2014). Table 4.3 shows the fatty acid percentages of *Vachellia tortilis* leaves. *Vachellia tortilis* leave contains 39 % SFA, 3.7 % MUFA and 58 % PUFA, respectively. It was noted that the PUFA: SFA was lower (1.5), which is beneficial to human health than that recommended by Mapiye *et al*. (2011). Table 4.4 shows fatty acid percentages of the experimental diet. Saturated fatty acid of these diets ranges from 13.6 - 14.0 %, MUFA, ranges from 24 - 24.5 %. PUFA was consistently across dietary treatments (62 %). The inclusion levels of *Vachellia tortilis* slightly reduce PUFA: SFA from 4.6 to 4.4 %.

Table 4.1 Nutrient composition of experimental diets

	Vachellia tortilis inclusion level (g/kg DM)					
Component	0	30	60	90	120	150
DM (g/kg)	946	935	899	940	897	896
GE (MJ/kg)	17.6	17.5	17.3	17.3	17.4	17.2
Ash (g/kg DM)	90	90	90	89	89	86
CP(g/kg DM)	144	140	142	146	146	148
EE(g/kg DM)	114	104	109	114	113	116
Starch (g/kg DM)	318	321	305	274	239	225
ADF (g/kg DM)	137	136	139	125	125	127
NDF (g/kg DM)	303	313	321	339	348	356
Lysine (g/100g DM)	10.3	10.1	10.1	10.5	11.0	10.7
Threonine(g/100g DM)	7.2	7.2	7.5	8.0	7.5	8.0
Methionine(g/100g DM)	5.0	4.3	4.5	4.3	4.8	5.0
CT (mg/kg DM)	-	2.3	3.1	5.1	6.6	7.9
BD(ml/g DM)	1.59	1.47	1.51	1.53	1.47	1.47
WHC (g_{water}/g_{feed} DM)	1.36	1.34	1.62	1.59	1.52	1.63
Calcium (g/kg DM)	10.1	15.4	16.8	17.3	17.2	19.8
Phosphorus (g/kg DM)	7.3	9.7	10.1	10.7	10.3	11.9
Magnesium (g/kg)	9.8	8.9	10.1	10.1	9.3	10.1
Potassium (g/kg)	8.8	9.0	9.3	10.2	10.4	11.1
Sodium (g/kg)	2.3	5.3	10.2	10.1	9.8	12.3
Zinc (mg/kg)	90.8	91.1	88.3	81.2	82.3	78.2
Copper (mg/kg)	8.9	7.8	9.14	8.15	7.23	7.31
Manganese (mg/kg)	124	111	109	124	123	128
Iron (mg/kg)	146	201	304	338	344	359

BD- bulk density, DM - dry matter; NDF- neutral detergent fibre; ADF- acid detergent fibre; ADIN - acid detergent insoluble nitrogen, CT-condensed tannins; WHC- water holding capacity; S.D – standard deviation

Table 4.2: Fatty acid composition (% of total fatty acids) of *Vachellia tortilis* leaves

Fatty acid	(% of total fatty acids)
SFA	
C14:0 (Myristic)	0.58
C15:0 (Pentadecanoic)	0.54
C16:0 (Palmitic)	24.2
C18:0 (stearic)	10.4
C20:0 Arachidic	0.66
C22:0 (Behenic)	0.36
C23:0 (Lignoceric)	0.13
C24:0 Lignoceric	1.08
MUFA	
C16:1 (Palmitoleic)	0.19
C18:1n9c (Oleic)	3.37
C20:1n9 (Eicosenoic)	0.03
C24:1n9 (Nervonic)	0.11
PUFA	
C18:2n6c (lauric)	11.4
C18:3n3 (α-linolenic)	44.3
C20:2n6 (Eicosadienoic)	0.11
C20:3n3 (Eicosatrienoic)	1.15
C22:2n6 (Docosadienoic)	0.38
C22:6n3 (DHA)	0.36
SFA	38.6
MUFA	3.71
PUFA	57.7
PUFA:SFA	1.5
n-6 (omega-6)	11.9
n-3 (omega-3)	45.8
(n-6)/(n-3)	0.26

MUFA= Monounsaturated fatty acids, PUFA= Polyunsaturated fatty acids, SFA= Saturated fatty acids

Table 4.1: Fatty acid composition (% of total fatty acids) of experimental diets

Experimental diets						
Fatty acid	0	30	60	90	120	150
[x]SFA						
C14:0	0.08	0.08	0.09	0.09	0.09	1.0
C15:0	0.03	0.04	0.04	0.03	0.03	0.04
C16:0	10.5	10.6	10.4	10.2	10.3	10.3
C18:0	2.51	2.67	2.71	2.82	2.97	3.00
C20:0	0.11	0.13	0.13	0.13	0.14	0.14
C22:0	0.19	0.21	0.22	0.22	0.24	0.23
C23:0	0.00	0.00	0.00	0.00	0.00	0.04
C24:0	0.15	0.16	0.16	0.18	0.19	0.20
[y]MUFA						
C16:1	0.12	0.13	0.13	0.12	0.12	0.14
C18:1n9c	23.9	24.1	24.0	24.0	23.9	23.9
C18:1n9t						
C20:1	0.20	0.20	0.20	0.20	0.19	0.19
C24:1	0.03	0.03	0.02	0.02	0.02	0.02
[z]PUFA						
C18:2n6c	59.6	58.7	58.8	58.7	58.2	58.0
C18:3n3	2.35	2.66	2.82	3.0	3.36	3.42
C20:2n6	0.06	0.05	0.05	0.05	0.05	0.04
C20:3n3	0.01	0.15	0.14	0.10	0.11	0.12
C22:2n6	0.06	0.05	0.05	0.05	0.05	0.05
C22:6n3	0.03	0.04	0.03	0.04	0.03	0.05
[x]SFA	13.6	13.8	13.8	13.7	14.0	14.0
[y]MUFA	24.3	24.5	24.0	24.4	24.2	24.3
[z]PUFA	62.2	61.7	61.9	62.0	61.8	61.7
PUFA:SFA	4.6	4.5	4.5	4.5	4.4	4.4
n-6	59.8	58.8	58.9	58.8	58.3	58.1
n-3	2.4	2.9	3.0	3.1	3.5	3.6
(n-6)/(n-3)	24.9	20.6	19.7	18.7	16.6	16.2

[x]Saturated fatty acids; [y]Monounsaturated fatty acids; [z]Polyunsaturated fatty acids

4.3.1 Growth performance

All pigs used in the experiment were clinical healthy throughout the experiment. Growth performances of pigs are shown in Table 4.5. Average daily feed intake was significantly ($P< 0.05$) affected by time on feed. A linear increase ($P< 0.05$) in ADFI was observed in Week 1, 2 and 3, respectively. In Week 4, a quadratic decrease ($P<0.03$) in ADFI with inclusion of *V. tortilis* leaf meal was observed. There was a linear ($P< 0.05$) decrease in ADG and G: F with increase inclusion levels of *V. tortilis* leaf meal in the diet. Overall ADFI increase linearly (Y= 0.061025x + 2.155240; $P< 0.05$) with inclusion level of *V. tortilis* in the diet, while both ADG and G: F decreased linearly (Y = -0.006291x + 0.925080; Y= -0.006299 + 0.414923; $P< 0.05$) with increase inclusion level of *V. tortilis*.

4.3.2 Carcass characteristics

The relationship between graded levels of *V. tortilis* leaf meal and carcass characteristics is shown in Table 4.6. Inclusion of *V. tortilis* linearly reduced ($P < 0.05$) slaughter weight (SL), warm carcass weight (WW), cold carcass weight (CW), back-fat thickness (BFT) and linear increased ($P < 0.05$) cooler shrink (CS). The inclusion level of *V. tortilis* had no effect ($P> 0.05$) on dressing percentage (DP), pH_{45} and $temperature_{45}$.

Table 4.3 Performance of pigs fed on graded levels of *Vachellia tortilis* leaf meal

Variable	Week	*Vachellia tortilis* inclusion level (g/kg DM)						SEM	Significance	
		0	30	60	90	120	150		Linear	Quadratic
ADFI	1	2.37	2.77	3.02	2.53	2.61	2.75	0.17	NS	NS
	2	3.09	3.02	3.17	3.05	2.74	2.91	0.17	NS	NS
	3	2.91	3.06	3.16	2.79	3.02	3.18	0.17	NS	NS
	4	2.98	3.23	3.58	3.32	2.67	2.78	0.17	NS	***
Over-all ADFI		2.83	3.02	3.19	2.91	2.76	2.86	0.15	***	NS
ADG	1	1.01	0.88	0.97	0.82	0.90	0.68	0.09	***	NS
	2	1.05	0.71	0.94	0.78	0.75	0.75	0.09	***	NS
	3	1.01	0.92	0.82	0.87	0.64	0.60	0.09	***	NS
	4	1.23	0.10	0.90	0.87	0.57	0.40	0.09	***	NS
Over-all ADG		1.08	0.88	0.91	0.84	0.72	0.61	0.07	***	NS
G:F	1	0.44	0.32	0.32	0.31	0.35	0.25	0.03	***	NS
	2	0.35	0.23	0.32	0.27	0.28	0.26	0.03	***	NS
	3	0.36	0.31	0.27	0.31	0.21	0.20	0.03	***	NS
	4	0.41	0.32	0.27	0.27	0.23	0.16	0.03	***	NS
Over-all G:F		0.39	0.29	0.29	0.29	0.27	0.22	0.02	***	NS

ADFI= average daily feed intake, ADG = average daily gain, DM = dry matter, G: F = gain: feed ratio, NS = not significant ($P > 0.05$); SEM = standard error of mean.

Table 4. 4 Relationship between graded levels of *V. tortilis* leaf meals and carcass characteristics of finishing pigs

Variable	*Vachellia tortilis* leaf meal inclusion level (g/kg DM)									
	0	30	60	90	120	150	SEM	Sig	Equations	R^2
Slaughter weight (kg)	90.3	85.4	86.3	84.5	82.1	79.6	1.03	***	y= -0.504x + 88.8	0.30
Warm weight	70.3	68.4	68.4	68.3	66.2	64.8	0.94	***	y= -0.146x + 69.8	0.30
Cold carcass weight	68.7	66.6	66.5	64.2	62.2	62.7	0.94	***	y= -0.183x + 68.2	0.34
Dressing percentage	76.6	78.1	76.9	79.1	78.5	78.3	1.21			NS
Cooler shrink (%)	2.32	2.55	2.77	2.78	3.0	3.24	0.06	***	y= 0.055 x + 2.4	0.74
BFT (mm)	11.9	11.6	10.7	9.9	9.3	8.5	0.31	***	y = -0.199x + 12.0	0.68
pH (45 min)	6.58	6.6	6.7	6.6	6.7	6.7	0.17			NS
Temperature (45 min)	35.7	35.0	34.1	35.6	34.6	34.8	0.54			NS

BFT= back-fat thickness, SEM = standard error of means, R^2= R-square value, Sig= significant level, NS= not significant, *** $P < 0.001$.

4.4 Discussion

Performance data of the current study are in agreement with previous work reported elsewhere (Khanyile *et al*., 2014). The linear increase in daily feed intake in pigs fed incremental levels of *Vachellia tortilis* leaf meal may be attributed to a decrease in nutrient availability owing to the presence of polyphenolic compounds and fibre. Phenolic compounds, such as proanthocyanidins, depress feed intake (Makkar, 2003), precipitate with protein (Mueller-Harvey, 2006) and possible damage the mucosal lining of the digestive track when the level in the feed are exceed 10 % (Halimani *et al*., 2005). High fibre levels of *Vachellia* leaf meal could have also limited the intake of digestible energy in the diet causing dissatisfaction (Martens *et al*., 2009). The observation that ADG was linearly reduced with increased levels of *V. tortilis* leaf meal in the diet could be ascribed to the reduction of maximum intake of nutrients caused by the binding effect of phenolic and the increased of fiber which eventual reduce the gut capacity of pigs (Martens *et al*., 2012). As a result of linear decreased in optimum intake of nutrients with increase inclusion levels of *Vachellia tortilis* leaf meal, it was expected that the G: F ratio woud follow a similar trend. This is in agreement with the results presented by Martins *et al*. (2009), who reported reduction in mass gain and poor gain: feed ratio of finishing pigs fed on two levels (5 and 10 %) of olive leaves. The recently report by Mukombo *et al*. (2014) also demonstrated increased in ADFI and reduction in ADG and G: F of finishing pigs fed on *Moringa* oleifera leaf meal.

The linearly decrease in slaughter weight, warm carcass weight and cold mass could be ascribed to the reduction in ADG when *V. tortilis* leaf meal increased in the diet. This is consistent with previous report by Khanyile *et al.* (2016) that reported a linear decrease in carcass characteristics of pi gs fed on incremental levels of *Vachellia tortilis* leaf meal. The observed linear reduction in back-fat thickness could be ascribed to low intake of digestible energy associated with leaf meal diets, consequently, resulting in low fat depot in the carcasses. This is in agreement with report by Paiva-Martins *et al*. (2009) who reported a reduction of back-fat thickness of carcasses from pigs fed on diet supplemented with Olive leaves. The linear increase in cooler shrink with increase inclusion of *V. tortilis* leaf meal in the diet could be ascribed to the decreased of back-fat thickness in carcasses from pigs fed on incremental levels of *V. tortilis* leaf meal. The lean carcasses lose more water in the first 24 hours to 48 hours (Schweihofer, 2011). Despite the observed decreased in cooler shrink, all reported values were within the acceptable range (between one and five percent) reported by Schweihofer (2011).

4.5 Conclusions

Increasing the inclusion of *V. tortilis* was accompanied by an increase in the negative effect in ADG, G: F, slaughter weight, warm carcass weight, cold weight and back-fat thickness. It will be worth investigating the effect of graded levels of *V. tortilis* on physicochemical properties of pork.

4.6 References

Abdulrazak, S.A., Orden, E. A., Ichinohe, T., Fujhara, T., 2000. Chemical composition, phenolic concentration and in vitro gas production characteristics of selected *Acacia* fruits and leaves. *Journal of Animal Science, 7, 935-940*.

AOAC. International (2002). Official methods of analysis (17th edition), Arlington. Virgina, USA: Association of Official Analytical Chemists Inc. Loss on drying (moisture) at 95 - 105ºC for feeds. AOAC Official Method 934.01.

Chimonyo, M., Bhebhe, E., Dzama, K., Halimani, T.E., Kanengoni, A. 2005. Improving smallholder pig production for food security and livelihood of the poor in Southern Africa. *African Crop Science Conference Proceedings, 7, 569-573*.

Dube, J.S., Reed J.D, Ndlovu, L.R. 2001. Proanthocyanidins and other phenolics in *Acacia* leaves of Southern Africa. *Animal Feed Science and Technology*, *91, 59-67.*

Folch, J., Lees, M., Sloane-Stanley, G.H. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biology and Chemistry, 226, 496-509.*

Gardner, J.A.A., Dunkin, A.C., Lloyd, L.C. 1990. Pig production in Australia.Butterwoths, Sydney.

Goering, H.K., Van Soest, P.J., 1970. Forage fibre analysis (apparatus, reagents, procedures and some applications), Agricultural Handbook No. 379.

Agricultural Research Service, US Department of Agriculture, Washington, DC.

Halimani, T. E., Ndlovu, L. R., Dzama, K., Chimonyo, M., Miller, B. G., 2005. Metabolic response of pigs supplemented with incremental levels of leguminous *Acacia karroo, Acacia nilotica* and *Colophospermum mopane* leaf meals. *Journal Animal Science, 81, 39-45.*

Halimani, T.E., Ndlovu, L.R., Dzama, K., Chimonyo, M., Miller, B.G., 2007. Growth performance of pigs fed on diets containing *Acacia karroo, Acacia nilotica* and *Colophosperamum mopane* leaf meals. *Animal Science, 81, 39-45*.

Hansen, M. J., A. Chwalibog, A. H. Tauson, E., Sawosz. 2006. Influence of different fibre sources on digestibility and nitrogenand energy balances in growing pigs. *Archives of Animal Nutrition, 60, 390–401*.

Khanyile, M. Ndou, S.P., Chimonyo, M. 2014. Influence of *Acacia tortilis* leaf meal based diets on growth performance of pigs. *Livestock Science, 167, 211-218*.

Khanyile, M., Ndou, S.P., Chimonyo, M. 2016. Influence of *Acacia tortilis* leaf meal-based diet on serum biochemistry, carcass characteristics and internal organs of finishing pigs. *Animal Production Science,* http:// dx. Doi. Org / 10. 1071/ AN 15182.

Lee, C.M., Trevino, B., Chaiyawat, M. 1996. A simple and rapid solvent extraction method for determining total lipids in fish tissue. *Journal of AOAC international, 79. (2), 487-49.2*

Lindberg, J.E. 2014. Fiber effects in nutrition and gut health in pigs. *Journal of Animal Science and Biotechnology, 5:15.*

Makkar, H. P. S., 2003. Effects and fate of tannins in ruminant animals, adaptation to tannins and strategies to overcome detrimental effects of feeding tannins-rich feeds. *Small Ruminant Research, 49, 241-256*.

Mapiye, C., Chimonyo, M., Dzama, K., Strydom, P., Muchenje, V., Marufu, M.C., 2009. Nutritional status, growth performance and carcass characteristics of Nguni steers supplemented with *Vachellia karroo* leaf-meal. *Livestock Science*, *126, 206-214.*

Mapiye, C., M. Chimonyo, K. Dzama, P. E. Strydom, V. Muchenje. 2010. Meat quality attributes of Nguni steers supplemented with *Acacia karroo* leaf-meal. *Meat Sci*ence, *8, 621-627*.

Mapiye, C., Chimonyo, M., Marufu, M. C., Dzama, K., 2011. Utility of *Acacia karroo* for beef production in Southern Africa smallholder farming systems: A review. *Animal Feed Science and Technology, 164, 135-146.*

Martens, S.D., Tiemann, T.T., Bindelle, J., Peters, M., Lascano, C.E. 2012. Alternative plant protein sources for pigs and chickens in the tropics-nutritional value and constraints: a review. *Journal of Agriculture Rural Development Tropical and Subtropical*, *113, 101-123.*

Mueller-Harvey, I., 2006. Unraveling the conundrum of tannins in animal nutrition and health. *Journal of Science Food and Agriculture, 86, 2010-2037.*

Mukombo, F.E., Maphosa, V., Hugo, A., Nkukwana, T.T., Mabusela, T.P., Muchenje, V. 2014. Effect of *Moringa Oleifera* leaf meal on finisher pig

growth performance, meat quality, shelf life and fatty acid composition of pork. *South African Journal of Animal Science*, *44, 388-400*.

Ndou, S.P., Khanyile, M., Chimonyo, M. 2015. Growth performance and nutrition-related serum metabolites in growing pigs fed on *Acacia tortilis* leaf meal. *Livestock Science, 182, 22-27*.

NRC. 2012. Nutrient Requirements of Swine, 11th rev. ed. Natl Acad. Press. Washigton DC, USA.

Paiva-Martins, F.P, Barbosa, S., Pinheiro, V., Mourão, J.L., Monteiro, D.O. 2009. The effect of olive leaves supplementation on the feed digestibility, growth performances of pigs and quality of pork meat. *Meat Science, 82, 438-443.*

Peterson, A. D., Baumgardt, B. R**.** 1971. Food and energy intake of rats fed diets varying in energy concentration and density. *Journal of Nutrition, 101, 1057-1068*.

SAS, 2008. SAS User's Guide: Statistics, Version9.1. SAS Institute, Cary, NC, USA.

Schweihofer, J.P. 2011.Carcass dressing percentage and cooler shrink vary among species and type of animals. Michigan State University.

Reed, J. D., McDowell, R. E., Van Soest, P.J., Horvath, P.J. 1982. Condensed tannins: a factor limiting the use of cassava forage. *Journal of Science and Food Agriculture, 33**,** 213-220.*

Robertson, J.B., Van Soest, P.J., 1977. The fibre estimation in concentrates feedstuffs. In: Proceeding of the 69 Meeting of the American Association of

Animal Science. University of Wisconsin, Madison, MI, USA, 23–27 July 1977.

Robertson, J. A. Eastwood, M. A. 1981. A method to measure the water-holding properties of dietary fibre using suction pressure. *British Journal of Nutrition, 46, 247-255.*

Chapter 5: Pork quality attributes from pigs fed on graded levels of *Vachellia tortilis* leaf meal

Abstract

The present study determined physicochemical attributes of pork from Large White x Landrace male pigs aged 14 weeks and weighing 63.6 ± 0.73 kg (mean ± SD) fed graded levels (0, 30, 60, 90, 120 and 150 kg DM) of *Vachellia tortilis* leaf meals. Following 32 days of feeding, all pigs (86 ± 1.03 kg) were slaughtered and *Longissimus dorsi* muscles were taken between 5th and 13th rib for meat quality analyses. There was a quadratic response ($P < 0.05$) ultimate meat pH (pH_{24}), Hue and Warner Bratzler Shear Force (WBSF) with increasing levels of *Vachellia tortilis*. There was a quadratic response ($P < 0.05$) in intramuscular fat (IMF) and pork redness (a^*) with increasing in *V. tortilis* in the diet. There was a linear response ($P < 0.05$) in pork moisture, lightness (L^*), yellowness (b^*), Chroma and cooking loss and linear response ($P < 0.05$) in muscle protein with increasing *V. tortilis* in the diet. Dry matter percentage and ash of pork were not affected by the inclusion levels of V. *tortilis* leaf meal. The current study has revealed that low levels of *V. tortilis* leaf meal improves quality of pork.

Keywords: Chroma, cooking loss, Hue, Intramuscular fat *Longissimus dorsi,* Warner

(Under review in *Meat Science*)

5.1 Introduction

Successful attempt to reduce high feed cost has been achieved through utilization of non-conventional feed resources such as *Vachellia* leaf meal in feeding livestock in the tropics (Mapiye *et al*., 2010; Ndou *et al.,* 2015). The constituent diet has a major influence on the physico-chemical and nutritional quality of pork (Dugan *et al.,* 2004). Colour of pork and intramuscular fat have a profound influence on the acceptability of meat by consumers, while tenderness and juiciness influence the palatability of cooked pork (Glitsh, 2000; Verbeke *et al.,* 2005).

Vachellia tortilis, former *Acacia tortilis*, belong to the family *Mimoseae I* and is widely distributed in the tropical and subtropical countries (Kayalangalilwa *et al.*, 2013). Besides being used for feeding livestock, *Vachellia species* have multipurpose roles including fencing, firewood and treatment of various human diseases (Mapiye *et al.,* 2010; Gowri *et al.,* 2011). *Vachellia tortilis* leaves are high in protein and omega-3 fatty acids, and contain moderate mineral contents (Khanyile *et al.,* 2014). Despite its high nutritional composition, the utilization in feeding pigs is limited by the presence of polyphenolics and high fibre (Dube *et al.,* 2001). Feeding animals with *V. karroo* leaf meal rich in phenolic compounds has been reported to increase omega-3 fatty acids, conjugated linoleic acid concentrations and produce meat with a redder colour and high protein content compared to meat from animals fed grasses (Mapiye *et al., 2011*).

Polyphenolic compounds act as natural antioxidants (Luhucky *et al.,* 2010). Currently, there has been a growing interest in the use of natural antioxidants to reduce the occurrence of cardiovascular problems and some cancer which are associated with synthetic antioxidants (Hayes *et al.,* 2010). Natural antioxidant plays a major role in the retardation of lipid oxidation, and improve colour stability of meat. The effect of feeding *V. tortilis* leaf meal on physicochemical properties of pork have not been investigated. The objective of current study was, therefore, to determine pork quality from pigs fed on graded levels of *V. tortilis* leaf meal based diets. It was hypothesized that feeding pigs with increasing levels of V. *tortilis* leaf meal has no effect on pork quality.

5.2 Materials and methods

5.2.1 Study site

The study site has been described in chapter 4.

5.2.2 Pigs, diets and housing

Pigs, diets and housing have been described in chapter 4.

5.2.3 Collection of pork samples

Following 24 hours of chilling the carcasses at 2 ºC, *Longissimus thoracisi* muscle (LT) samples were taken between the 5th and last 13th rib, vacuum packed in plastic bags and transported to the Meat Science Laboratory at Stellenbosch University,

South Africa, under chilled conditions. Meat sample were kept at -20 ºC pending meat quality analysis.

5.2.4 Meat quality analyses

5.2.4.1 Determination of pH

Ultimate pH (pH_{24}) were measured using portable pH meter equipped with a spear tip electrode and an automatic thermometer compensator probe (CRISON pH25, CRISON instrument SA, Spain). The pH meter electrode was rinsed using distilled water between each measurement to avoid treatment contamination.

5.2.4.2 Colour

Pork colour at 24 h was determined using CIE Lab meter (Colour –guide 45º/0º colorimeter (BYK-Gardner GmbH, Gerestried, Germsny) with 20 mm diameter measurement area and illuminant D65-d light, 10º standard observer. Briefly, LD muscle samples of each pig measuring 5 cm in diameter and with a thickness of 2.5 cm were cut and allowed to bloom for 30 min at 4ºC before measurement. Colour measurements were taken from three locations on the cut surface of individual LD muscle. The colour scale was expressed as L* (lightness), a* (redness), b* (yellowness) (EIC, 1976). Hue angle, which describes the fundamental colour of a substance, and Chroma, which describes the saturation of a colour, are calculated as: $\tan^{-1}$ (b*/a*) and $(a^{*2}+ b^{*2})^{0.5}$, respectively (Minolta, 1993).

5.2.4.3 Cooking loss

Freshly cut steaks from *LT* muscle were weighed and placed in clearly labeled thin-walled plastic bags. Samples were then cooked at 75ºC for 60 min. The samples were cooled in chill room at 4ºC until equilibrated. Water accumulated in the bag during cooking was removed. Pork samples from bags were gently blotted dry and weighed to determine final weight. Cooking loss was calculated using the following formula:

% Cooking loss = [(weight BEFORE - weight AFTER) / (weight BEFORE)] * 100

5.2.4.4 Shear force

The tenderness of *LT* sample was determined using Warner Bratzler Shear force (WBSF) (Honikel, 1998). The force in Newton (N) required to shear each block perpendicular to its grain of the muscle fiber was determined using an Instron Universal Testing Machine (UTM, model 2519-107 Universal Testing Instrument Instron, Canton, MA) fitted with a Warner-Bratzler shearing device. The Instron had a load cell of 2 kN and crosshead speed of 200 mm/ min. Pork samples from the cooking loss measurement were used. Visible connective tissue membranes (perimysial) in thes samples were removed. At least, six 1 × 1 × 2 cm blocks were cut from *LT* muscle samples, which were used for the determination of cooking loss. The blocks were cut such that the muscle fibres ran parallel to the longitudinal axis of these blocks. The average of six values for all blocks was used as the Warner Bratzler Shear Force of the sample.

5.2.5 Proximate analysis of *LT* samples

Frozen *LT* samples were properly thawed at 4ºC overnight. Excess fats were removed from samples using scalpel. Forty-eight hours after slaughter a 50g sample was minced for determination of moisture, dry matter, ash and protein as described by AOAC (2002, method.934.01; 942.05 and 992.15). Intramuscular fat (IMF) content was extracted according to the method described by Lee *et al*. (1996). Briefly, IMF content was determined on a 5 g homogenized *LD* muscle sample using a 1:2 chloroform/methanol solution for fat extraction. The IMF of the muscle samples content were then determined according to AOAC (1995; method 920.39).

5.2.6 Statistical analyses

The polynomial regression procedure (PROC REG) of SAS (2009) was used to determine linear and quadratic effects of feeding increasing dietary levels of *V. tortilis* on pork quality.

5.3 Results

5.3.1 Chemical attributes

Chemical attributes of *LT* muscle from pigs fed graded levels of *V. tortilis* leaf meal is shown in Table 5.1. A linear response ($P < 0.05$) in Ultimate pH (pH_{24}) with increasing levels of *V. tortilis* leaf in the diet was observed. There was a linear

responce ($P < 0.05$) in moisture % and muscle protein with increasing levels of *V. tortilis*. Dry matter and ash percentage were not affected by *V. tortilis* inclusion. There was a quadratic response ($P < 0.05$) in Intramuscular fat with increasing levels of *V. tortilis* leaf meal. Using the segmented regression, it was revealed that the propotion of IMF stated to decrease when the leaf meal was included beyond 90g/kg DM.

5.3.2 Physical attributes

Physical attributes of LD muscle from pigs fed graded levels of *V. tortilis* leaf meal is shown in Table 5.2. There was a linear response in muscle lightness (L*), muscle yellowness (b*), chrome and cooking loss with the increasing levels of *V. tortilis* leaf meal in the diet. Quadratic response ($P < 0.05$) in muscle redness (a*) were observed with increase in levels of *V. tortilis* leaf meal in the diet. There was a quadratic response ($P < 0.05$) in hue and WBSF with the increase levels of *V. tortilis* leaf meal in the diet. Using segmented regression, the colour redness a* stated to decrease when the *V. tortilis* leaf meal was included beyond 60g/kg DM while the maximum Hue angle was achieved at 150g/kg DM *V. tortilis* leaf meal.

Table 5.1 Chemical attributes of *Longissimus dorsi* muscle from pigs fed on graded levels of *Vachellia tortilis* leaf based-diets

	Vachellia tortilis inclusion (g/kg)							Significance		Equations	R^2
Trait	0	30	60	90	120	150	S.E.M	Linear	Quad		
pH24	5.3	5.4	5.4	5.4	5.5	5.6	0.02	***	NS	y= 0.06658x + 0.032808	0.32
Moisture (%)	75.0	74.1	74.1	74.6	74.4	74.6	0.26	***	NS	y = -0.1638x + 74.807656	0.30
DM (%)	25.0	26.0	26.0	25.4	25.6	25.4	0.26	NS	NS		
Ash (%)	1.08	1.08	1.14	1.10	1.11	1.13	0.06	NS	NS		
IMF (%)	2.67	2.83	2.98	2.60	2.10	1.98	0.1	***	***	y= $-0.009450x^2$ + 0.07910x + 2.6981	0.50
CP (%)	20.7	22.1	22.3	22.7	20.7	21.6	0.45	***	NS	y = 0.337423x + 20.973504	0.30

DM= Dry matter, CP= Crude protein, IMF= Intramuscular fat, NS= Not significant, pH 24= ultimate pH, Quad= Quadratic, SEM= standard error of means, Sig = Significant level, ***P <0.001.

Table 5.2 Physical attributes of *Longissimus dorsi* muscles from pigs fed on graded levels of *Vachellia tortilis* leaf meal based diets

	Vachellia tortilis inclusion (g/kg)							Significance		Equations	R^2
Parameter	0	30	60	90	120	150	S.E.M	Linear	Quad		
L*	53.4	52.1	51.7	50.9	51.9	49.2	0.42	***	NS	y= -0.162419x + 52.996362	0.42
a*	5.04	4.80	4.72	3.97	2.89	2.12	0.07	NS	***	$y=-0.013227x^2-0.00239x+5.031741$	0.50
b*	11.9	11.1	10.9	10.2	9.74	9.02	0.12	***	NS	y= -0.177613x + 11.831652	0.64
Chroma	10.8	10.1	10.4	10.3	9.54	9.44	0.23	***	NS	y= -0.069857 + 10.721685	0.40
Hue	67.1	66.5	66.2	68.6	73.4	76.8	0.43	NS	***	$y= 0.07962x^2 + 0.512311x + 670339$	0.51
CL (%)	35.2	33.9	32.5	33.0	32.7	32.2	0.36	***	NS	y= -0.433078x + 35.081406	0.31
WBSF (N)	30.5	31.1	32.1	36.2	40.9	43.8	0.45	***	NS	y= 0.233556x + 30.15801	0.42

a^*= redness of meat, b^*= Yellowness of meat, CL= cooking loss, L^*= lightness of meat, N= neuton, Quad= Quadratic, SEM = standard error of means, Sig= significant level, WBSF= Warner Bratzler Shear Force.

5.4 Discussion

The current study tested for linear and quadratic effects of feeding increasing dietary levels of *V. tortilis* on pork quality. The ultimate pH (pH 24) in the current study showed a quadratic increase with increase inclusion levels of leaf meal up to a point where there was no further increase. The quadratic increase in pH_{24} observed with increased levels of *V. tortilis* leaf meal could be ascribed to the decrease in maize proportion of the experimental diets, consequently reducing the glycogen. Maize is the major source of glycogen for pigs. Phenolic compounds in *V. tortilis* leaf meal could have also reduce carbohydrates digestibility there by lowering level of glycogen. Phenolics have strong H–donating activity (Muchuweti *et al.*, 2007) and reported to act as natural antioxidant (Mapiye *et al.,* 2011). These results are in agreement with the findings by Lee *et al*. (2012), who reported an increase in ultimate pH of thigh meat from broilers fed 1.0% dietary gallic acid and linoleic acid. The quadratic increase in ultimate pH was within the normal range (5.7-6.1), indicating no signs of pale, soft and exudative (PSE) or dark, firm and dry (DFD) as described by de Vries and van der Wal (1992).

The linear decrease in moisture content of *LT* muscle as *V. tortilis* leaf meal increased could be attributed to quadratic increase in ultimate pH. Water loss in the muscle is mainly influence by denaturation of protein caused by high ultimate pH (Bruce *et al.*, 2004; Huff-Lonergan and Lonergan, 2005; Wu *et al*., 2014). The other cause of decrease in moisture loss could be the initial increase in IMF percentages, which was observed from 30-90g/kg inclusion level before a

quadratic decrease. Lower percentages of IMF observed from 120-150g/kg DM slightly increases the moisture content of pork. Moisture content of meat is negatively correlated (r=-0.99) with IMF content (Shuler *et al.,* 1970; Shields *et al.,* 1983; Ramsey *et al.,* 1990). Despite the observed decrease in the moisture content of the LD muscle with increase in *V. tortilis* inclusion, values obtained in the current study were within the acceptable range (72%-75%), reported by Kim *et al*. (2006). Moisture content influences both firmness and cooking loss during cooking procedure (Pearce *et al.,* 2011).

The quadratic decrease of IMF percentages could be ascribed to phenolic compounds in experimental diets. In the current study the inclusion of *V. tortilis* leaf meal initial increase IMF percentages up to 60 g/kg inclusion level before a quadratic decline observed from 90g/kg DM inclusion level. At lower inclusion levels of leaf meals, pigs are known to increase the intake of feed to compensate nutrients deficiencies caused by fibre and phenolic compounds (Martens *et al.,* 2012). The quadratic decrease of IMF beyond 90g/kg inclusion levels was expected as the results of increase of phenolic compounds, which reduces feed intake (Halimani *et al*., 2005; Khanyile *et al.*, 2014) and consequently, reduce fat deposition in the body and muscle tissue (Paiva-Martins *et al.*, 2009). Intramuscular fat is largely influenced by diet (Wood *et al*., 2004). Even though experimental diets were formulated to render similar levels of energy and crude protein, the availability of phenolic compounds in leaf meal could have resulted in imbalance energy to protein ratio through complexing effect. Despite the quadratic

decrease in IMF in the current study, all reported values were within the normal range (2-4%) required to provide meat with marbling characteristics appreciated by consumers (Verbeker *et al.*, 1999). The quadratic decrease in IMF percentages of LD muscle in the current study agrees with Paiva-Martins *et al.* (2009) who reported decreased in IMF content of pork from pigs fed on varying levels of *Olive* leaves. Zhang et al. (2009) reported a reduced intramuscular fat (IMF) with early feeding (28th to 57th day of life) of 0.1% sea buckthorn extract to crossbred pigs. A decrease in the total muscle fat has also been reported in pork from pigs fed on varying levels of *Moringa oleifera* (Mukumbo *et al.,* 2014). Lipids plays vital role during cooking, and influences tenderness, juiciness and flavor of meat (Elmore and Mottram, 2009).

The observed linear decrease in lightness (L*) could be ascribed to the quadratic increase in ultimate pH (Bidner *et al.,* 2004), quadratic decrease in intramuscular fat content and linear decrease in moisture content of LD muscles (Bruce *et al.,* 2004;) with increasing levels of *V. tortilis* leaf meal. Despite the decrease in L* value with increasing inclusion levels of leaf meal, all the values were within the normal range indicating no signs of pale, soft and exudative (PSE) or dark, firm and dry (DFD) meat.

The quadratic decrease in a* could have been caused by increase in ultimate pH observed with increasing inclusion level of *V. tortilis* leaf meal in the diet. Higher ultimate pH values are known to cause dry surface on meat thereby reducing the

rate of oxymyoglobin, consequently yielding meat dark colour (Priolo *et al.,* 2001; Holmer *et al.*, 2009). High ultimate pH affect the colour stability of fresh meat by reducing enzyme activity and the rate of oxygenation (Govindarajan, 1973). Reducing enzymes are necessary to convert metmyoglobin back to oxymyoglobin (Ledward, 1992). Another plausible explanation of the reduction in enzymes activity could be precipitating effects of phenolic compounds present in *V. tortilis* leaf meal, since enzymes are protein in nature (Makkar*,* 2003). The high ultimate pH causes a dry surface, which inhibits the penetration of oxygen into the meat and thus slows down the oxygenation process (Pettigrew and Esnaola, 2001). Other explanation for the decrease in redness could be through metal chelation properties of phenolic compounds present in leaf meals. Phenolic compounds are known to complex with iron, which plays critical role in the formation of red colour pigmentation. The current finding that redness decrease as the leaf meal increase is in agreement with Mukumbo *et al*. (2014) who demonstrated a significant decrease in redness in LD muscle from pigs fed on Moringa leaf meal. The increase in redness (a*) of meat from Nguni cattle supplemented with *V. karroo* has been reported by Mapiye *et al*. (2010).

The decreased in colour parameter b* could be ascribed to decreasing maize proportion, which could have resulted in reduced beta-carotene content of the leaf meal diets. The decrease b* in the current study contradicts with Mukumbo *et al*. (2014) and Wapi *et al*. (2013) who reported an increase in b* values with increasing *Moringa oleifera* leaf meal in pig diets, which could relate to differences in

nutritional and phenolics composition of the species and the fact that the maize proportion was not reduced with inclusion level of *Moringa* leaf meal.

The quadratic increase in Hue in pork sample from pigs fed on *V. tortilis* leaf meal implies that the meat colour was more intense, this could be ascribed to the presence of phenolic compounds, which are reported to possess antioxidative properties (Muchuweti *et al.,* 2007; Moyo *et al.,* 2011). This is in agreement with the report by Mukumbo *et al*. (2014) who observed increased in Hue angle of pork from pigs fed on *Moringa oleifera* leaf meal. The quadratic increase in Hue suggest that *V. tortilis* leaf meal did not deteriorate the redness of meat (Hoffman *et al.,* 2012) and could prolong the acceptability of pork. Pork colour has profound impact on consumer's decision to purchase meat (Ngapo *et al.,* 2007; Gracia and de Magistris, 2013).

The observed linear decrease in cooking loss with increasing inclusion levels of *Vachellia* leaf meal indicates that the meat was able to retain juices during cooking. This could be attributed to the quadratic decrease in the intramuscular fat and linear decrease in moisture of pork from observed with increasing inclusion of V*. tortilis* leaf meal. Levels of fat in the meat have a profound effect on the cooking loss (Yu *et al*., 2005). The melting of fat and denaturation of the protein during cooking, general causes the release of chemically bound water (Vieira *et al.*, 2009). Ngambu *et al.* (2013) also reported a decrease in cooking loss of meat from goats supplemented with *V. karroo*.

The quadratic increase in Warner Bratzler Shear Force (WBSF) observed with increasing *V. tortilis* leaf meal inclusion could be ascribed to decrease in intramuscular fat content of pork from pigs fed on incremental levels of *Vachellia* leaf meal based diet. Meat texture largely depends on the size of muscle fibre, amount of connective tissue, and quantity of IMF (Joo, *et al.,* 2013). Intramuscular fat act as lubricants of muscle fibre during cooking, consequently, reducing shear force and increasing tenderness of meat (Luchak *et al.,* 1998). IT is general known that high WBSF value is associated with poor consumer acceptability of meat product. All values of WBSF observed with increase *V. tortilis* leaf in the diet were within the acceptable range showing no indication of poor tenderness.

5.5 Conclusions

Vachellia tortilis leaf meal could be included up to 120 g/kg DM without compromising pH_{24}, moisture, DM, ash, CP, L*,b* and CL of pork. If the rednes (a*), Hue and IMF of pork is the main intrest, V. tortilis leaf meal can be included at a rate of 60, 90, and 150g/kg DM respectively. The decrease in IMF percentages warrant further research on the effect *V. tortilis* of fatty acid profile of pork.

5.6 References

AOAC. 1995. Official methods of analysis 16th Ed. Association of official analytical chemists. Washington DC, USA.

AOAC. International 2002. Official methods of analysis (17th edition), Arlington. Virgina, USA: Association of Official Analytical Chemists Inc. Loss on drying (moisture) at 95 - 105ºC for feeds. AOAC Official Method 934.01.

Bidner, B.S., Ellis, M., Brewer, M.S, Campion, D., Wilson, E.R., Mckeith, F.K. 2004. Effect of ultimate pH on the tenderness of retail meat. *Meat Science, 59, 139-154.*

Bruce, H.L., Stark, J.L., Beilken, S.L. 2004. The effects of finishing diet and postmortem ageing on the eating quality of the *m. longissimus thoracis* of electrically stimulated Brahman steer carcasses. *Meat Science, 67, 261-268.*

CIE, 1976. Colorimetry, 2nd ed. Commission International de l'Eclairage, Vienna, Switzerland.

de Vries, A.G. van der Wal, P.G. 1992. Breeding for quality. In: E Puolane and D.I. Demeyer, (Eds).

Diaz, M.T., Velasco, S., Caneque, V., Lauzurica, S., Ruiz de Huidobro, F., Perez, C. 2002. Use of concentrate feeding or pasture for fattening Lambs and its effect on carcass and meat quality. *Small Ruminant Research, 43, 257-268*.

Dube, J.S., Reed J.D., Ndlovu, L.R. 2001. Proanthocyanidins and other phenolics in *Acacia* leaves of Southern Africa. *Animal Feed Science and Technol*ogy, *91, 59-67.*

Dugan, M.E.R., Aalus, J.L., Utaro, B. 2004. Nutritional manipulation of pork quality: Current opportunities. *Advances in Pork Production, 15, 237-243*.

Elmore, J. S., Mottram, D. S. 2009. Flavour development in meat. In J. R. Kerry, and D. Ledward (Eds.), Improving the sensory and nutritional quality of fresh meat (pp. 111-146). CRC Press: Woodhead Publishing Limited.

Glitsh, K. 2000. Consumer perceptions of fresh meat quality: Cross-national comparison. *British Food Journal*, *102, 177-194*.

Goering, H.K., Van Soest, P.J., 1970. Forage fibre analysis (apparatus, reagents, procedures and some applications), Agricultural Handbook No. 379. Agricultural Research Service, US Department of Agriculture, Washington, DC.

Govindarajan, S. 1973. Fresh meat colour. *CRC Critical Reviews in Food Technology, 1*, *117-140*.

Gowri, S.S., Pavitha, S., Vasantha, K. 2011. Free radical scavenging capacity and antioxidant activity of young leaves and bark of *Acacia nilotica* (L.) Del. *Int. Journal of Pharmacology Science, 3, 160-164.*

Gracia, A., de Magistris, T. 2013. Preferences for lamb meat: A choice experiment for Spanish consumers. *Meat Science*, *95, 396- 402*.

Hayes, J. E., Allen, P., Brunton, N., O'Grady, M.N., Kerry, J. P. 2010. Phenolic composition and in-vitro antioxidant capacity of four commercial phytochemical products: Olive leaf extract (Olea europaea L), lutein, sesamol and ellagic acid. *Food Chemistry, 126, 948-955.*

Hoffman, L.C., Vermaak, A., Muller, N. 2012. Physical and chemical properties of selected beef muscles infused with a phosphate and lactate blend. *South African Journal of Animal Science, http//dx.doi.org/10.4314/sajas. v42i4.1*.

Holmer, S.F., Mckeith, R.O., Boler, D.D., Dilger, A.C., Eggert, J.M., Petry, D.B., Mckeith, F.K., Jones, K.L., Killefer, J. 2009. The effect of pH on shelf-life of pork during aging and simulated retail display. *Meat Science, 82, 86-93.*

Honikel, K.O. 1998. Reference methods for the assessment of physical characteristics of meat. *Meat Science*, *49, 447-457.*

Huff-Lonergan, E., Lonergan, S.M. 2005. Mechanisms of water holding capacity of meat: the role of post mortem biochemical and structural changes. *Meat Science, 71, 194-204*.

Joo, S.T., Kim, G.D., Hwang, Y.H., Ryu, Y.C. 2013. Control of fresh meat quality through manipulation of muscle fiber characteristics. *Meat Science, 95, 828-836.*

Kayalangalilwa, B., Boatwright, J.S., Daru, B.H., Maurin, O., van der Bank, M. 2013. Phylogenetic position and revised classification of *Acacia s.l.* (Fabaceae: Mimosoideae) in Africa, including new combinations in *Vachellia* and *Senegalia*. *Botany Journal of Linnean Society*, *172, 500-523.*

Khanyile, M. Ndou, S.P., Chimonyo, M. 2014. Influence of *Acacia tortilis* leaf meal based diets on growth performance of pigs. *Livestock Science, 167, 211-218.*

Khanyile, M., Ndou, S.P., Chimonyo, M. 2016. Influence of Acacia tortilis leaf meal-based diet on serum biochemistry, carcass characteristics and internal

organs of finishing pigs. *Animal Production Science, http:// dx. Doi. Org / 10. 1071/ AN 15182.*

Kim, H., Song, Y., Jin, S., Kim, S., Kang, Y., Lee, S., Chowdappa, R., Ha, J., Kang, S. 2006. The effect of change in meat quality parameters on pig *Longissimus dorsi* muscle by the addition of fermented persimmon shell diet. *Asian-Australian Journal of Animal Science, 2, 286-291.*

Ledward, D.A. 1992. Colour of raw and cooked meat. In Johnston, D.E., Knight M.K & Ledward, D.A. *The chemistry of muscle-based foods* (pp *128-144*). Cambridge: Royal Society of Chemistry.

Lee, C.M., Trevino, B., Chaiyawat, M.1996. A simple and rapid solvent extraction method for determining total lipids in fish tissue. *Journal of Association of Official Analytical Chemists International, vol. 79, no 2*.

Lee, K.H., Jung, S., Kim, H.J., Kim II.S., Lee, J.H., Jo, C. 2012. Effect of dietary supplementation of the combination of gallic and linoleic acid in thigh meat of broilers. *Asian-Australian Journal of Animal Science, 25, 1641-1648*.

Luchak, G. L., Miller, R. K., Belk, K. E., Hale, D. S., Michaelsen, S. A., Johnson, D. D., West, R. L., Leak, F. W., Cross, H. R., Savell, J. W. 1998. Determination of sensory, chemical and cooking characteristics of retail beef cuts differing in intramuscular and external fat. *Meat Science, 50, 55-72.*

Luhucky, R., Nuernberg, K., Kovac, L., Bucko, O., Nuermberg, G. 2010. Assessment of the antioxidant potential of selected plant extracts-In vitro and in vivo experiments on pork. *Meat Science, 85, 779-784.*

Makkar, H. P. S. 2003. Effects and fate of tannins in ruminant animals, adaptation to tannins and strategies to overcome detrimental effects of feeding tannins-rich feeds. *Small Ruminant Research*, *49, 241-256.*

Mapiye, C., M. Chimonyo, K. Dzama, P. E. Strydom, V. Muchenje. 2010. Meat quality attributes of Nguni steers supplemented with *Acacia karroo* leaf-meal. *Meat Science*, *8, 621-627.*

Mapiye, C., Chimonyo, M., Marufu, M. C., Dzama, K. 2011. Utility of *Acacia karroo* for beef production in Southern Africa smallholder farming systems: A review. *Animal Feed Science Technology, 164, 135-146*.

Martens, S.D., Tiemann, T.T., Bindelle, J., Peters, M., Lascano, C.E. 2012. Alternative plant protein sources for pigs and chickens in the tropics-nutritional value and constraints: a review. *Journal of Agriculture Rural Development Tropical and Subtropical*, *113, 101-123.*

Minolta, 1993. Precise colour communication. Minolta Camera, Ltd., Ramsey, New Jersey.

Moyo, B., Masika, P.J., Hugo, A., Muchenje, V. 2011. Nutritional characterization of Moringa (*Moringa oleifera* Lam.) leaves. *African Journal of Biotechnology, 10, 12925-12933.*

Muchuweti, M., Kativa, E., Mapure, C.H., Chidewe, C., Ndhlala, A.R., Benhura, M.A.N. 2007. Phenolic composition and antioxidant properties of some species. *American Journal of food technology, 2 (5), 414-420*.

Mukumbo, F.E., Maphosa, V., Hogo, A., Nkukwane, T.T., Mabusela, T.P., Muchenje, V. 2014. Effect of Moringa Oleifera leaf meal on finisher pig

growth performance, meat quality, shelf life and fatty acid composition of pork. *South African Journal of Animal Sc*ience, *44, 388-400.*

Ndou, S.P., Khanyile, M., Chimonyo, M. 2015. Growth performance and nutrition-related serum metabolites in growing pigs fed on *Acacia tortilis* leaf meal. *Livestock Science*, *182, 22-27*.

Ngambu, S., Muchenje, V., Marume, U. 2013. Effect of Acacia karroo supplementation on growth, ultimate pH, colour and cooking losses of meat from indigenous Xhosa Lop-eared Goats. *Asian-Australian Journal of Animal Science, 26, 128-133.*

Ngapo, T. M., Martin, J. F., Dransfield, E. 2007. International preferences for pork appearance: I. Consumer choices. *Food Quality and Pref*erence, 18, 26-36.

NRC. 2012. Nutrient Requirements of Swine, 11th rev. ed. Natl Acad. Press. Washigton DC, USA.

Paiva-Martins, F.P, Barbosa, S., Pinheiro, V., Mourão, J.L., Monteiro, D.O. 2009. The effect of olive leaves supplementation on the feed digestibility, growth performances of pigs and quality of pork meat. *Meat Science, 82, 438-443.*

Pearce, K.L., Rosenvold, K., Andersen, H.J., Hopkins, D.L. 2011. Water distribution and mobility in meat during the conversion of muscle to meat and ageing and the impacts on fresh meat quality attributes-a review. *Meat Science*, *89, 111-124*.

Peiretti, P.G., Gai, F., Brugiapaglia, A., Mussa, P.P., Mussa G.M. 2015. Fresh meat quality of pigs fed diets with different fatty acid profiles and

supplemented with red wine solids. *Food Science and Technol*ogy, *35 (4), 633-642.*

Pettigrew, J.E., Esnaola, M.A. 2001. Swine nutrition and pork quality: A review. *Journal of Animal Science, 79(E. Suppl.): E316-E342*.

Peterson, A. D., Baumgardt, B. R**.** 1971. Food and energy intake of rats fed diets varying in energy concentration and density. *Journal of Nutrition, 101, 1057-1068.*

Priolo, A., Micol, D, Agabriel, J. 2001. Effects of grass feeding systems on ruminant meat colour and flavour. A review. *Animal Research, 50,185-200.*

Priolo, A., Micol, D., Agabiel, J., Prache, S., Dransfield, E. 2002. Effect of feeding of grass or concentrate feeding systems on lamb carcass and meat quality. *Meat Science*, *62, 179-185*.

Ramsey, C. B., Tribble, L. F., C. Wu, C., Lind, K.D. 1990. Effects of grains, marbling and sex on pork tenderness and composition. *Journal of Animal Science, 68, 148-159*.

Reed, J. D., McDowell, R. E., Van Soest, P.J., Horvath, P.J. 1982. Condensed tannins: a factor limiting the use of cassava forage. *Journal of Science Food and Agriculture, 33**,** 213-220.*

Robertson, J.B., Van Soest, P.J. 1977. The fibre estimation in concentrates feed stuffs. In: Proceeding of the 69 Meeting of the American Association of Animal Science. University of Wisconsin, Madison, MI, USA, 23-27 July 1977.

SAS, 2009. SAS User's Guide: Statistics, Version9.1. SASInstitute, Cary, NC, USA.

Shields, R. G., Mahan, D.C., Graham, P.L. 1983. Changes in swine body composition from birth to 145 kg. *Journal of Animal Sci*ence, *57, 43-58.*

Shuler, R. O., Pate, T. D., Mandigo, R. W., Lucas, L. E. 1970. Influence of confinement, floor structure and slaughter weight on pork carcass characteristics. *Journal of Animal Sci*ence, *31, 31-45.*

Verbeke, W., De Smet, S., Vackier, I., Van Oeckel, M. J., Warnants, N., Van Kenhove, P. 2005. Role of intrinsic search cues in the formation of consumer preferences and choice for pork chops. *Meat Science*, *69, 343-354.*

Verbeke, W., Oeckel van, M. J., Warnants, N., Viaene, J., Boucqué, Ch. V. 1999. Consumer perception, facts and possibilities to improve acceptability of health and sensory characteristics of pork. *Meat Science, 53, 77-99.*

Vieira, C., Diaz, M.Y., Martinez, B., Garcia-Cachὰn, M.D. 2009. Effect of frozen storage conditions (temperature and length of storage) on microbial and sensory quality of rustic crossbred beef at different stages of aging. *Meat Science, 83, 398-404*.

Walker, P., Rhubart-Berg, P., McKenzie, S., Kelling, K., Lawrence, R.S. 2005. Public health implications of meat production and consumption. *Public Health Nutrition*, *8, 348-356.*

Wapi, C., Nkukwana, T.T., Hoffman, L.C., Dzama, K., Pieterse, E., Mabusela, T., Muchenje, V. 2013. Physico-chemical shelf-life indicators of meat from broilers given *Moringa oleifera* leaf meal. *South African Journal of Animal Science, 43 (Supp. 1), 43-47*.

Wood, J.D., Nute, G.R., Richardson, R.I., Whittington, F.M., Southwood, O., Plastow, G., Mansbridge, R., da Costa, N., Chang, K.C. 2004. Effect of breed, diet and muscle on fat deposition and eating quality in pigs. *Meat Science, 67, 651-667.*

Wu, G., Farouk, M.M., Clerens, S., Rosenvold, K. 2014. Effect of beef ultimate pH and large structural protein changes with aging on meat tenderness. *Meat Science, 98, 637-645.*

Yu, L.H., Lee, E.S., Jeong, J.Y., Paik, H.D., Choi, J.H., Kim, C.J. 2005. Effect of thawing temperature on the physicochemical properties of pre-rigor frozen chicken breast and leg muscles. *Meat Science*, *71, 375-382*.

Zhang, Z., Xia, L. L., Chen, X., Zhao, W., Wang, P., Su, J., Li, Y. 2009. Effects of eally feeding sea buckthorn extracts on growth performance, meat quality, serum leptin levels and leptin mRNA expression of adipose tissue in swine. Chine Journal of Animal Nutrition, 21, 734-740.

Chapter 6: Fatty acid composition, atherogenicity and thrombogenicity indices of pork from pigs fed on *Vachellia tortilis* leaf meal

*(*Under review in *Journal of Food Composition and Analysis)*

Abstract

The objective of the current study was to determine fatty acid composition, nutritive value, atherogenecity and thrombogenecity indices of pork from 48 Large White x Landrace male pigs aged 14 weeks averaging 63 ± 0.93 (SD) kg BW) fed graded levels (0, 30, 60, 90, 120 and 150 g/kg DM) of *Vachellia tortilis* leaf meal. Following 32 days of feeding, all pigs were slaughtered and *Longissimus dorsi* muscles were taken between 5th and 13th rib and kept at -20℃ for the determination fatty acid composition. Overall, the total fatty acids content, proportions of individual and total saturated fatty acids (SFA), C24:1, C18:2*n*6 and total *n*-6 polyunsaturated fatty acids (PUFA), *n*-6/*n*-3 ratio, atherogenic index (AI) and thrombogenic index (TI) decreased linearly (P<0.001) with increasing levels of *V. tortilis* leaf meal in the diet. There was a quadratic increase (P<0.001) in monounsaturated fatty acids (MUFA). Total *n*-3 PUFA, total PUFA and nutritive value index increased and then decreased in a quadratic (P <0.001) fashion with increase inclusion of *V. tortilis* leaf meal. The highest proportions of C18:3*n*-3, C20:5*n*3, C20:3*n*3 and total *n*-3 PUFA were obtained at 70, 65, 62 and 68 g/kg DM of V*. tortilis*, respectively (P<0.05). The largest proportions of C20:2*n*-6, C20:3*n*-6 and C22:2*n*-6 were reached at 91, 93 and 67 g/kg DM of *V. tortilis* leaf meal. It was concluded that inclusion of *V. tortilis* leaf meal up to 70g/ kg DM in pig diets increased nutritive

index, proportions of *n*-3 PUFA and total MUFA, and reduced total SFA, total *n*-6 PUFA, *n*-6/*n*-3 PUFA ratio, atherogenic and thrombogenic indices in pork.

Key words: atherogenic index, consumer health, Longissimus dorsi, proanthocyanidins, *n*-6: *n*-3 ratio.

*(*Under review in *Journal of Food Composition and Analysis)*

6.1 Introduction

Over the 20th century, there has been growing interest in the limitation of saturated fatty acids (SFA) and increase intake of polyunsaturated fatty acids (PUFA) in human nutrition due to high incidences of various diseases including diabetes type II and coronary heart diseases (Simopoulos, 2011; Vahmani *et al*., 2015). Overall, research has been towards the reduction of fat intake, as well as bringing the ratio of PUFA to SFA (P: S) in meat above 0.45 to improve human health (Wood *et al*., 2008). The applicability of this ratio is, however, questionable as it assumes that all SFA and PUFA are biologically equivalent to one another and act antagonistically. In addition, there is insufficient evidence linking SFA intake with cardiovascular disease (CVD), possibly because of the neutral effects of 18:0 and/or positive effects of 14:0 and 16:0 on high-density lipoprotein (HDL-C) (Hunter *et al.*, 2010) known to favourably impact on CVD risk factors (Natarajan *et al*., 2010; Vannice and Rasmussen, 2014). As such, it is important to pay more attention on the correlation between SFA and consumer health, as it is likely dependent on specific fatty acids (FA) and other constituents in foods that contain SFA (de Oliveira Otto *et al.*, 2012).

The *omega-6* (*n*-6) to omega-3 ratio is another nutritional index that has been used to assess the healthfulness of food products with a ratio of 4:1 being considered optimal (Simopoulos, 2008; Dept-Health-and-Human-Services, 2014). The effectiveness of *n*-6:*n*-3 at predicting the reduced risks of CVD, however, falls short because it does not differentiate α-linolenic acid (ALA) from the metabolically more active eicosapentaenoic and docosahexaenoic acid (EPA/DHA) (Griffin, 2008). Individual FA in the same class may have different biological effects, some of which may be antagonistic (Dugan *et al.,* 2015). Current evidence suggests that it is the actual intake of specific FA that are associated with many different endpoints (Flock *et al*., 2013). There is, therefore, no scientific rationale for the continued recommendation of a specific ratio of P:S or *n*-6/*n*-3 in assessing the quality of foods (Aranceta and Pérez-Rodrigo, 2012).

There is evidence that diet has a major influence on fatty acid profile of pork (Maw *et al*., 2003; Dugan *et al*., 2004; Mas *et al*., 2010). In pigs, dietary fatty acids are absorbed unchanged from the intestines unlike in ruminants, where dietary fatty acids are biohydrogenated in the gut (Wood *et al*., 1999). The fatty acid composition in pigs, therefore, closely reflects dietary fatty acid composition (Turner *et al*., 2014). Saturated and monounsaturated fatty acids in pigs are also synthesized *de novo* and, are not easily influenced by diet compared to polyunsaturated fatty acids such as linoleic (18:2*n*-6) and α-linolenic (18:3*n*-3), which cannot be synthesized endogenously (Doreau *et al*., 1997; Enser *et al*., 2000). Feeding diets that are rich in α-linolenic acid increases levels of the *n-3*

PUFA (Wood *et al*., 2008) which lowers thrombogenicity (Enser *et al*., 2000) defined as the tendency of a material in contact with the blood to produce a thrombus, or clot (López and Chen, 2009).

To reduce feed cost and competition of feed ingredients between livestock and humans, utilization of *Vachellia* leaf meal in feeding livestock in Southern Africa has been explored in ruminants (Mapiye *et al*., 2011; Marume *et al*., 2012) and non-ruminants (Halimani *et al*., 2007; Khanyile *et al*., 2014; Ndou *et al.,* 2015; Hlatini *et al*., 2016; Khanyile *et al*., 2016). *Vachellia* leaves have high levels of proteins, minerals, vitamins and proanthocyanidins (Dube *et al.,* 2001; Mokoboki *et al.*, 2005) and 18: *n*-3 (Mapiye *et al*., 2011). The presence of proanthocyanidins and high fibre levels in *Vachellia* leaf meal decrease feed intake, growth rate and back fat thickness in pigs (Khanyile *et al*., 2016). *Vachellia* leaf meal has, however, been reported to improve 18:3*n*-3 and *n-6/ n-3* ratio in beef (Mapiye *et al*., 2011). So far, the effect of *Vachellia tortilis* leaf meal on fatty acid profile of pork is still unknown. Therefore, the present study was undertaken to determine the effect of feeding *Vachellia tortilis* leaf meal on fatty acid composition, atherogenesity and thrombogenecity indices of pork.

6.2 Materials and methods

6.2.1 Study site

The study site has been described in Section 4.1.

6.2.2 Pigs and housing

Pigs and housing has been described in Section 4.1.

6.2.3 Diets

Diets have been described in Section 4.1

6.2.4 Determination of pork fatty acid profiles

The fat from a 1 g sample of raw LD muscle homogenate was extracted using a 2:1 (v/v) chloroform: methanol solution (Folch *et al*., 1957), which contained 0.01 % butylated hydroxytoluene (BHT) as an antioxidant. The samples were homogenized in the extraction solvent for 30 secs using a polytron mixer (WiggenHauser D-500 Homogenizer, fitted with a standard shaft 1, speed setting D). Heptadecanoic acid (C17:0) was used as an internal standard (catalogue number H3500, Sigma-Aldrich, Gauteng, South Africa). A 250-µL sub-sample of the extracted lipids was subsequently transmethylated at 70 ºC for 2 hours using 2 mL of a 191 (v/v) methanol: sulphuric acid solution as the transmethylating agent. After allowing the resultant mixtures to cool at room temperature, the fatty acid methyl esters (FAME) were extracted with water and hexane. Following separation of the distilled water and FAME- containing hexane fluids, the top hexane layer was transferred to a spotting tube and dried under nitrogen. Fifty µL hexane was then added to each dried FAME sample, of which 1 µL was injected into the gas chromatograph. The FAMEs were analyzed using a Thermo TRACE 1300 series gas-chromatograph (Thermo Electron Corporation, Milan, Italy) equipped with a

flame-ionization detector, a 30 m TR-Fame capillary column with an internal diameter of 0.25 mm and a 0.25 µL film (Cat. No. HY260M142P, Anatech, Cape Town, South Africa) and a run time of *ca*. 40 mins. The following oven temperature settings were utilized: initial temperature of 50 ºC (maintained for 1 min) and final temperature of 240 ºC attained after three ramps (initial increase at a rate of 25 ºC/min until a temperature of 175 ºC was reached; thereafter an immediate increase at a rate of 1.5 ºC/min to reach 200 ºC and maintenance of this temperature for a minimum of 2 min). The injector temperature was set at 240 ºC and the detector temperature at 250 ºC. The hydrogen gas flow rate was 40 mL/min. The FAME of sample was identified by comparing the retention times with those of a standard FAME mixture (Supelco^{TM} 37 Component FAME mix, Cat no, 47885-U, Sulpeco, USA), with results being expressed as mg fatty acid/g meat.

The saturation (saturated FA: unsaturated FA ratio, S:P), atherogenic index (AI), and thrombogenic index (TI) was calculated using the formulae proposed by Ulbricht and Southgate (1991), as follows: AI = (C12:0 + 4 × C14:0 + C16:0)/[ΣMUFA + Σ(n-6) + Σ(n-3)];TI = (C14:0 + C16:0 + C18:0)/[0.5 × ΣMUFA + 0.5 × Σ(n-6) + 3 × Σ(n-3) + Σ(n-3)/Σ(n-6)]; where MUFA = monounsaturated FA; PUFA = polyunsaturated FA. Nutritive value was calculated using the formulae proposed by Fernảndez *et al*. (2007) as follows: [(C18:0+C18:1n9c)/C16:0]

6.2.5 Statistical analyses

The polynomial regression procedure (PROC REG) of SAS (2009) was used to determine linear and quadratic effects of feeding increasing dietary levels of *V. tortilis* on fatty acid composition and nutritive value, atherogenic index and thrombogenic indices. The piecewise regression (broken-stick) analysis was conducted using the NLIN procedure(SAS,2009) to estimate the threshold value at which the inclusion level of *V. tortilis* leaf meal caused a particular fatty acid or group of fatty acids to be constant or to decrease as the incremental levels of *V. tortilis* increased. The model used was as follows:

$Y_i = \gamma_o + \gamma_1 + \gamma_2 (Ix_c) (x_i - x_c) + \varepsilon_i$,

Using parameters (γ_o, γ_1, γ_2) and the x_c, the two segmented simple regression functions were;

$Y_j = \gamma_o + \gamma_1 (x_i)$, for $x_i \leq x_c$; and

$Y_k = Y_o + (\gamma_1+\gamma_2) x_i$, for $x_i \geq x_c$

Where;

Y_i is the response variable when *V. tortilis* leaf meal inclusion level constrains fatty acids; Y_j is the response variable before *V. tortilis* leaf meal inclusion level constrains fatty acids; Y_k is the response variable when *V. tortilis* leaf meal exceeds the optimum inclusion level;

$Y_o = \gamma_o - \gamma_2 x_c$; when $x_i = 0$;

γ_o is the intercept or minimum Y_i when $x_c < 0$;

γ_1 is the rate of change of Y_i when $x_i < x_c$;

γ_2 is the rate of increase in Y_i when $x_i > x_c$;

x_i is the inclusion level of *V. tortilis* in the diet;

x_c is the optimum inclusion level beyond which a fatty acid is constrained by elevated *V. tortilis* leaf meal in the diets; and

Ix_c is a dummy variable with value 0 when $x_i < x_c$ and 1 when $x_i \geq x_c$.

The significance threshold was set at $P < 0.05$

6.3 Results

6.3.1 Fatty acid composition, atherogenic index, thrombogenic index and nutritive value of pork

Fatty acid composition, atherogenic index, thrombogenic index and nutritive value of *Longissimus dorsi* muscles from pigs fed graded levels of *V. tortilis* leaf meal are given in Table 6.1 and Table 6.2. Overall, total fatty acid content, individual and total SFA, C24:1, C18:2*n*-6, C18:3*n*-6, C20:4*n*-6, total *n*-6 PUFA, C22:6*n*-3, total PUFA, *n*-6: *n*-3 ratio, atherogenic and thrombogenic indices linearly decreased (*P* <0.001) with increasing inclusion levels of *V. tortilis* leaf meal. There were quadratic increases (*P* <0.001) in total MUFA, C16:1, C18:1*n*-9c, C18:1*n*-9t, C20:1 C18:3*n*-3, C20:3*n*-3, C20:5*n*-3, C22:2*n*-3, C20:2*n*-6, C20:3*n*-6, and nutritive value index (NVI) but further increase in leaf meal inclusion level led to a linear decrease (P <0.001). The ratio of PUFA/ SFA was not affected (*P* >0.05) by the inclusion levels of *V. tortilis* leaf meal.

6.3.2 Optimum inclusion of *V. tortilis* leaf meal

Table 6.3 shows segmented regression indicating the optimum inclusion levels of *V. tortilis* leaf meal beyond which the greatest proportions of a given fatty acid were attained. The highest proportions of C16:1 and C20:5*n*-3 were obtained at 65g/kg DM of *V. tortilis* inclusion level ($P < 0.05$). The greatest proportions of C18:1n9c and C181n9t were reached at 63g/kg DM of *V. tortilis* leaf meal inclusion level. For

C18:3*n*-3 and C20:3*n*-3, the largest proportions of were reached at 70 g/kg DM and 62 g/kg DM of *V. tortilis* leaf meal inclusion level, respectively. The greatest proportions of C20:2*n*-6, C20:3*n*-6 and C22:2*n*-6 were reached at 91, 93 and 67 g/kg DM of *V. tortilis* leaf meal inclusion levels. The largest proportions of total MUFA and total *n-3* PUFA were, respectively, obtained at 69 and 68 g/kg DM of *V. tortilis* leaf meal inclusion levels.

Table 6.1: Relationship between graded levels of *Vachellia tortilis* leaf meal and fatty acid (mg/g) composition of pork

	Vachellia tortilis inclusion (g/kg)							Significance		Equations	R^2
Fatty acid	0	30	60	90	120	150	S.E.M	Linear	Quadratic		0.65
C12:0	0.79	0.75	0.73	0.63	0.44	0.40	0.05	***	NS	y= -25.286x + 12.463	0.66
C13:0	0.53	0.53	0.40	0.34	0.31	0.34	0.02	***	NS	y= -42.826x + 14.143	0.57
C14:0	0.60	0.61	0.54	0.44	0.37	0.38	0.03	***	NS	y= -37.349x + 14.389	0.52
C15:0	0.30	0.25	0.32	0.29	0.22	0.22	0.01	***	NS	y= -47.654x + 6.5852	0.80
C16:0	5.29	5.25	5.25	4.31	4.06	3.90	0.08	***	NS	y= -13.278x + 38.585	0.74
C18:0	4.54	4.89	4.72	4.27	3.27	2.91	0.06	***	NS	y= -40.0558x + 14.342	0.81
C20:0	0.09	0.08	0.06	0.06	0.04	0.04	0.03	***	NS	y= -147.30x + 9.3422	0.86
C22:0	0.57	0.50	0.35	0.33	0.31	0.31	0.04	***	NS	y= -68.730x + 19.523	0.76
C24:0	0.83	0.82	0.70	0.62	0.52	0.46	0.03	***	NS	y= - 36.899x + 17.515	0.56
C16:1	0.34	0.39	0.43	0.46	0.34	0.27	0.01	***	***	$y=174.84x^2$ -136.30x+28.138	0.56
C18:1n9c	4.66	5.17	6.13	6.41	6.26	4.23	0.11	***	***	$y= 2.0555x^2$ – 21.942x + 59.390	0.57
C18:1n9t	0.16	0.17	0.18	0.18	0.12	0.11	0.03	***	***	$y= 1083.2x^2$ – 358.89x + 31.222	0.52
C20:1	0.17	0.20	0.22	0.21	0.14	0.13	0.03	***	***	$y= 862.96x^2$ – 327.54x + 32.279	
C22:1n9	0.18	0.22	0.23	0.24	0.22	0.20	0.04	***	NS	y=442.43x - 46.216	0.52
C24:1	0.42	0.45	0.46	0.42	0.36	0.33	0.01	***	NS	y= 124.16x + 31.447	0.73
C18:2n6	4.36	4.49	4.37	4.02	3.15	3.07	0.08	***	NS	y= -3.8664x + 14.495	0.50
C18:3n6	0.16	0.18	0.18	0.15	0.12	0.12	0.01	***	NS	y=-10.282x+6.7839	0.70
C18:3n3	0.20	0.21	0.20	0.18	0.15	0.13	0.01	***	***	$y=257.76x^2$ -131.98x+17.457	0.69
C20:2n6	0.26	0.26	0.30	0.24	0.20	0.18	0.01	***	***	$y=434.11x^2$ - 236.60x + 33.403	0.80
C20:3n6	0.80	0.82	0.71	0.46	0.44	0.39	0.03	***	***	$y=18.994x^2$ - 32.146x + 14.247	0.82
C20:3n3	0.22	0.23	0.22	0.16	0.15	0.13	0.04	***	***	$y=312.54x^2$ - 151.448x + 19.236	0.57
C20:4n6	0.79	0.86	0.87	0.61	0.58	0.46	0.03	***	NS	y= -7.8843x + 7.7624	0.58
C20:5n3	0.15	0.17	0.18	0.14	0.14	0.12	0.04	***	***	$y= 1362.5x^2$ + 478.73x + 42.891	0.74
C22:2n6	0.39	0.39	0.42	0.32	0.30	0.24	0.01	***	***	$y= 108.659x^2$ + 95.198 + 21.855	0.51
C22:6n3	0.20	0.21	0.30	0.34	0.32	0.30	0.01	***	NS	y= 47.754x – 6.5957	0.58

NS= not significant, R^2 = R-square, S.E.M = standard error of mean, ****P*< 0.0001

Table 6. 1: Relationship between *Vachellia tortilis* leaf meal with total group fatty acids and health indices

	Vachellia tortilis inclusion (g/kg)							Significance		Equations	R^2
Parameter	0	30	60	90	120	150	S.E.M	Linear	Quad		
SFA	13.6	13.7	13.1	11.3	9.55	8.97	0.13	***	NS	y= -0.5415x + 10.626	0.89
MUFA	5.94	6.58	7.65	7.93	7.45	5.28	0.11	***	***	$y= 1.4884x^2 + 19.806 + 66.854$	0.55
PUFA	7.48	7.82	7.76	6.63	5.39	5.15	0.11	***	NS	y= -2.3713x + 14.653	0.76
n-6 PUFA	6.77	6.99	6.85	5.80	4.79	4.47	0.09	***	NS	y= -4.3243x + 19.292	0.82
n-3 PUFA	0.77	0.82	0.91	0.83	0.74	0.67	0.01	***	***	$y= 74.751x^2 - 128.78x + 57.085$	0.56
PUFA/SFA	0.55	0.57	0.59	0.59	0.56	0.57	0.01	NS	***	$y= 237.69x^2 - 251.54x + 68.396$	0.53
n-6/n-3	8.88	8.55	7.56	7.02	6.44	6.64	0.17	***	NS	y= -4.6030x + 25.401	0.75
AI	3.45	3.43	3.11	2.55	2.09	2.12	0.15	***	NS	y= -8.6736x + 17.608	0.72
TI	13.2	13.4	13.3	11.4	10.1	9.80	0.12	***	NS	y= -4.7110x + 35.715	0.84
NV	5.42	5.88	5.90	5.76	4.82	4.00	0.08	***	***	$y= 0.8127x^2 - 9.8374x + 31.381$	0.55
TFA (mg/g fat)	27.0	28.1	28.5	25.8	22.4	19.4	0.25	***	NS	y= -0.5415x + 10.626	0.89

AI= atherogenic index, SFA= saturated fatty acid, MUFA= monounsaturated fatty acids, PUFA= polyunsaturated fatty acid, Quad= quadratic, NS= not significant, TFA= total fatty acid content, TI= thrombogenic index, NV= nutritive value, ***= P <0.001, **= P <0.05.

Table 6.3: Optimum *Vachellia tortilis* inclusion level indicating threshold value when fatty acids are constrained

Variable	γ_o		γ_1		γ_2		x_c		
	Estimates	*P*. Value	Estimates	*P*. value	Estimates	*P*. value	Estimates	Max Response	*P*. value
C16:1	0.345 (0.04)	0.264	0.067 (0.07)	0.29	-0.013 (0.03)	0.05	65	0.43	0.04
C18:1n9c	4.603 (1.03)	7.86	0.976 (2.20)	7.99	-0.212 (0.08)	2.56	63	5.73	0.65
C18:1nt	0.162 (0.002)	0.170	0.011 (0.004)	0.02	-0.002 (0.001)	0.002	63	0.85	0.02
C20:1	0.167 (0.001)	0.173	0.006 (0.002)	0.01	-0.0005 (0.0003)	0.001	60	0.19	0.01
C18:3n3	0.195 (0.002)	0.189	0.007 (0.003)	0.01	-0.001 (0.001)	0.002	70	0.21	0.04
C20:2n6	0.255 (0.004)	0.268	0.014 (0.006)	0.27	-0.002 (0.002)	0.03	91	0.28	0.04
C20:3n6	0.794 (0.015)	0.84	0.022 (0.022)	0.09	-0.004 (0.006)	0.01	93	0.84	0.04
C20:3n3	0.219 (0.003)	0.23	0.008 (0.006)	0.03	-0.002 (0.002)	0.005	62	0.23	0.04
C20:5n3	0.152 (0.003)	0.16	0.019 (0.006)	0.04	-0.004 (0.002)	0.003	65	0.18	0.01
C22:2n6	0.383 (0.02)	0.46	0.032 (0.04)	0.16	-0.006 (0.01)	0.04	67	0.43	0.38
MUFA	5.823 (0.33)	6.88	1.201 (0.53)	2.90	-0.205 (0.53)	0.31	69	7.58	0.04
n-3	0.760 (0.03)	0.87	0.098 (0.06)	0.28	-0.018 (0.02)	0.04	68	0.90	0.04

$Y_i = \gamma_o + \gamma_1 + \gamma_2 (Ix_c) (x_i - x_c) + \varepsilon_i$, when $(x_i > x_c) = 1$.

Where;
Y_i is the response variable when *V. tortilis* leaf meal inclusion level is constraining fatty acid
γ_o is the intercept or minimum Y_i when $x_c < 0$;
γ_1 is the rate of change of Y_i when $x_i < x_c$;
γ_2 is the rate of increase in Y_i when $x_i > x_c$;
x_c is the optimum level of inclusion beyond which fatty acids are constrained by increase in *V. tortilis* leaf meal in the diets.
Ix_c is a dummy variable with value 0 when $x_i < x_c$ and 1 when $x_i \geq x_c$. All measurements were considered significant at $P < 0.05$

6.4 Discussion

The observation that total fatty acid content, individual and total SFA decreased with increasing levels of *V. tortilis* leaf meal in pigs could be ascribed proanthocyanidins which are reported to reduce feed intake and pig growth (Khanyile *et al*., 2014), and impaired enzymatic activity thereby reducing digestibility, absorption and deposition of fats (Mandal and Ghosh, 2012; Khanyile *et al*., 2016). Another explanation could be related to decreases in starch and increases in neutral detergent fibre (NDF) and ether extracts EE contents with increasing levels of *V. tortilis* leaf meal in the diet. Thus, differences in total fatty acid contents could be associated with reduced lipogenic rates when feeding the low carbohydrate (starch) and high fibre (NDF) diets, an assumption that is supported by lower proportions of palmitic (C16:0) and stearic (C18:0) acids in the respective treatments. Palmitic and stearic acids are the first fatty acids synthetized from carbohydrate sources, and lower specific activities of key lipogenic enzymes (Gondret *et al*., 2014), which leads to reduction in fat content (Dugan *et al*., 2004; Duran-Montgé *et al*., 2010). Current findings are in accordance with the observation that dietary fat (EE) inhibits lipogenesis in isolated porcine adipocytes (Benmansour *et al*., 1991). An other possible cause could be the inhibition of lipase enzymes by proanthocyanidins, eventually reducing the digestibility and absorption of fatty acids (Aiura and de Carvalho, 2007; Mandal and Ghosh, 2012). Similarly, Mukumbo *et al*. (2014) reported reduction in intramuscular fat in *Longissimus dorsi* muscle from pigs fed on varying levels of *Moringa* leaf meal containing proanthocyanidins.

Saturated fatty acids such as lauric acid (12:0), myristic (14:0) and palmitic (16:0) are associated with development of cardiovascular disease in humans due to their low-density lipoprotein (LDL) (Fortin *et al*., 2005; Salter, 2013; Vahmani *et al*., 2015; Jiang and Xiong, 2016).

The observation that a majority of MUFA, including C18:1n9c (oleic acid) the main MUFA, increased quadratically could be ascribed to the increase in the proportion of stearic acid (C18:0) with increasing inclusion of *V. tortilis* leaf meal in the diet. Stearic acid is converted by Δ9 desaturase to oleic acid *de novo* (Smith *et al*., 2009). The reduction in C18:2n6 could have also contributed to the quadratic increase in C16:1. The C18:2*n*-6 is reported to inhibit Δ-9 desaturase activity subsequently reducing *de novo* synthesis of c9-16:1 from 16:0 (Nakamura *et al*., 2004). Oleic acid is reported to lower blood fat, improved HDL to LDL cholesterol ratios and prevent inflammatory processes in blood vessels (Miura *et al.,* 2013).

Given that individual and total *n*-6 PUFA were similar across diets, the observed linear decreases in *n*-6 PUFA in pork with increasing inclusion levels of *V. tortilis* leaf meal could be partly related to increased *n*-3 PUFA in pork. There is competition between omega-6 and omega-3 PUFA for the desaturation enzymes, with both fatty acid desaturase 1 and fatty acid desaturase 2, for example, preferring α-linolenic aid to linoleic acid (Indu and Ghafoorunissa, 1992; Abedi and Sahari, 2014; Simopoulos, 2016).

An other plausible explanation could be the astringent effect of proanthocyanidins which is reported to decrease feed intake (Khanyile *et al.,* 2014), thus reducing the intake of *n*-6 PUFA. Impaired enzymatic activity caused by proanthocyanidins (Aiura and de Carvalho, 2007; Mandal and Ghosh, 2012), could have also reduce the digestibility and absorption of lipids and consequently *n*-6 fatty acid.

The observation that a majority of individual and total *n*-3 PUFA increased in a quadratic fashion with inclusion levels of *V. tortilis* leaf meal could be related to the diet. The decrease in the deposition of individual and total *n*-3 PUFA at high inclusion levels of *V. tortilis* leaf meal could relate to increased intake of α-linolenic acid. Duran-Montge (2010) similarly reported that when dietary ALA levels in finishing pigs fed 10% fat/oil including flaxseed oil is low, body depositions of ALA were high (80-100%), and when dietary ALA levels were high, body depositions of ALA dropped to 60-70%. The author related that the drop in *n*-3 PUFA deposition to the extreme intake of α-linolenic acid. Once needs for phospholipid synthesis are met, surplus PUFA are likely diverted toward triacylglycerol synthesis and enter into pools more available for β-oxidation (Wood *et al*., 2003). Recent research has demonstrated that *n*−3 polyunsaturated (PUFA), have many properties that seem to promote human health and wellbeing (Calder, 2015; Dilzer and Park, 2012), particularly the long chain (LC) *n*-3 PUFA (eicosapentaenoic acid, EPA, 20:5*n*-3; docosapentaenoic acid, DPA, 22:5*n*-3; and docosahexaenoic acid, DHA, 22:6*n*-3)

have protective effects against heart disease, cancer, arthritis and other inflammatory diseases (Kaur *et al.*, 2011; Swanson *et al.,* 2012).

The linear decrease in atherogenecity index observed could be ascribed to linear decrease in SFA. All the reported values of atherogenecity index in the current study were below the 0.5, which is recommended by World Health Organization (WHO, 2003). The observation that graded levels of *V. tortilis* leaf linearly decrease thrombogenicity index could be ascribed high α-linolenic acid, which increases *n*-3 fatty acids involved in decreasing the thrombogenicity of blood (Enser *et al*., 2000). The linear increase in the nutritive value of pork could have resulted from a reduction in SFA and increases in PUFA, which in turn may have greatly reduced *n*-6/*n*-3 ratio.

Segmented regression showed that inclusion of *V. tortilis* leaf meal up to 70g/ kg DM increased nutritive index, proportions of *n*-3 PUFA and total MUFA, and reduced total SFA, total *n*-6 PUFA, *n*-6/*n*-3 PUFA ratio, atherogenic and thrombogenic indices in pork. This could be ascribed to reduced proportion of maize and wheat bran as the *V. tortilis* leaf meal increases in the diet. The proportion of *n*-3 PUFA was expected to increase due to high concentration of α-linolenic acid in the *V. tortilis* leaf meal. Further increase in leaf meal, however, led to increases levels of proanthocyanidins consequently reducing feed intake and digestibility of lipids. Pig's diets are always formulated to meet energy and protein or amino acids requirements with little emphasis on fatty acids. Previously,

recommendation of *Vachellia* leaf meal inclusion (100 and 130g/kg DM in finishing pigs (Halimani *et al.,* 2005; Khanyile *et al*., 2014) were based on performance and welfare of pigs without consideration of pork quality in general. It is, therefore, important for food enrichment to consider fatty acid requirements to address human health problems associated with fatty acid composition.

6.5 Conclusions

Results of the present study indicated that inclusion of *V. tortilis* leaf meal in finishing diet has the beneficial effect in increasing *n*-3 PUFA, MUFA, nutritive value index and reduce *n*-6: *n*-3 ratio, atherogenic and thrombogenic indices in pork. A largest proportions of individual and total *n*-3 PUFA were between 62 and 70g/kg, DM of *V. tortilis* leaf meal. The increased *n*-3 PUFA content of pork from pigs fed *V. tortilis* leaf provides the pig industry with an opportunity to provide value-added by healthful meat products for human consumption. It may be worth investigating the lipid stability of pork from pigs fed *V. tortilis* leaf meal. It is recommended that *V. tortilis* leaf meal be included at rate of 70 g/kg DM if quality and human health is the main objective and feed cost is not a crucial factor.

6.6 References

Abedi, E., Sahari, M.A. 2014. Long-chain polyunsaturated fatty acid sources and evaluation of their nutritional and functional properties. *Food Science and Nutrition, 2 (5), 443-463.*

Aiura, F.S., de Carvalho, M.R.B. 2007. Body lipid deposition in Nile tilapia fed on rations containing tannin. *Pesquisa Agropecuária Brasileira. 42, 51–56*.

AOAC. 2002. Official methods of analysis (17th edition), Arlington. Virgina, USA: Association of Official Analytical Chemists Inc. Loss on drying (moisture) at 95 - 105ºC for feeds. AOAC Official Method 934.01.

AOAC. 2003. Association of Analytical Chemists. Official Methods of Analysis, 14th edition. AOAC, Washington, DC, USA.

Aranceta J., Perez-Rodrigo, C. 2012. Recommended dietary reference intakes, nutritional goals and dietary guidelines for fat and fatty acids: a systematic review. *British Journal of Nutrition, 107,8-22*.

Benmansour, N. M., Demarne, Y., Lecourtier, M.J., Lhuillery, C. 1991. Effects of dietary fat and adipose tissue location on insulin action in young boar adipocytes. *International Journal of Biochemistry, 23,499-506.*

Calder, P. C. 2015. Functional roles of fatty acids and their effects on human health. *Journal of Parenteral and Enteral Nutrition*, *39, 18S-32S*.

de Oliveira Otto, M.C., Mozaffarian, D., Kromhout, D., Bertoni, A.G., Sibley, C.T., Jacobs Jr, D.R. Nettleton, J.A. 2012. Dietary intake of saturated fat by food source and incident cardiovascular disease: The Multi-Ethnic Study of Atherosclerosis. *American Journal of Clinical Nutrition, 96 (2), 397-404.*

Department of Health and Human Services. 2014. Food labeling: nutrient content claims; alpha-linolenic acid, eicosapentaenoic acid, and docosahexaenoic acid omega-3 fatty acids. *Federal Register, 79(81):23262-23273.*

Dilzer, A., Park, Y. 2012. Implication of conjugated linoleic acid (CLA) in human health. *Critical Reviews in Food Science and Nutrition, 52(6), 488-513*.

Doreau, M., Chilliard, Y. 1997. Digestion and metabolism of dietary fat in farm animals. *British Journal of Nutrition*, *78, S15-S35.*

Dube, J.S., Reed J.D, Ndlovu, L.R. 2001. Proanthocyanidins and other phenolics in *Acacia* leaves of Southern Africa. *Animal Feed Science Technol*ogy, *91, 59-67.*

Dugan, M.E.R., Aalus, J.L., Utaro, B. 2004. Nutritional manipulation of pork quality: Current opportunities. *Advances Pork Production, 15, 237-243.*

Dugan, M.E.R., Vahmani, P., Turner, T.D., Mapiye, C., Juårez, M., Prieto, Beaulieu, A.D., Zijlstra, Patience, J.F. Aalhus, J.L. 2015. Pork as a source of Omega-3 (*n*-3) fatty acids. *Journal of Clinical Medicine*, *4, 1999-2011, doi:10.3390/ jcm4121956.*

Duran-Montge, P., Realine, C.E., Barroeta, A.C., Lizardo, R.G., Esteve-Garcia, E. 2010. De novo fatty acid synthesis and balance of fatty acids of pigs fed different fat sources*. Livestock Science, 132, 157-164*.

Enser, M., Richardson, R.I., Wood, J.D., Gill, B.P., Sheard, P.R. 2000. Feeding linseed to increase the *n*-3 PUFA of pork: fatty acid composition of muscle, adipose tissue, liver and sausages. *Meat Science, 55, 201-212*.

Fernåndez, M., Ordonez, J.A., Cambero, I, Santos, C., Pin, C., de la Hoz, L. 2007. Fatty acid composition of selected varieties of Spanish dry ham related to their nutritional implications. *Food Chemistry, 101, 107-112*.

Folch, J., Lees, M., Sloane-Stanley, G.H. 1957. A simple method for the isolation and purification of total lipids from animal tissue. *Journal of Biology and Chemistry, 226, 497-509.*

Fortin, A., Robertson, W., Tong, A. 2005. The eating quality of Canadian pork and its relationship with intramuscular fat. *Meat Science*, *69, 297-305.*

Flock, M.R., Harris, W.S., Kris-Etherton, P.M. 2013. Long-chain omega-3 fatty acids: time to establish a dietary reference intake. *Nutrition Review*, 71,692-707.

Gondret, F., Louveau, I., Mourot, J., Duclos, M.J., Lagarrigue, S., Gilber, H., van Milgen, J. 2014. Dietary energy sources affect the pertition of body lipids and the hierarchy of energy metabolic pathways in growing pigs differing in feed efficiency. *Journal of Animal Science, 92, 4865-4877*.

Goering, H.K., Van Soest, P.J. 1970. Forage fibre analysis (apparatus, reagents, procedures and some applications), Agricultural Handbook No. 379. Agricultural Research Service, US Department of Agriculture, Washington, DC.

Griffin, B.A. 2008. How relevant is the ratio of dietary n-6 to n-3 polyunsaturated fatty acids to cardiovascular disease risk? Evidence from the OPTILIP study. *Current Opinion in Lipidology, 19 (1), 57-62.*

Halimani, T. E., Ndlovu, L. R., Dzama, K., Chimonyo, M., Miller, B. G. 2005. Metabolic response of pigs supplemented with incremental levels of leguminous *Acacia karroo, Acacia nilotica* and *Colophospermum mopane* leaf meals. *Journal of Animal Science, 81, 39-45.*

Halimani, T.E., Ndlovu, L.R., Dzama, K., Chimonyo, M., Miller, B.G. 2007. Growth performance of pigs fed on diets containing *Acacia karroo, Acacia nilotica* and *Colophosperamum mopane* leaf meals. *Animal Science, 81, 39-45*.

Hlatini, V.A., Khanyile, M., Zindove, T.J., Chimonyo, M. 2016. Feed intake and growth performance of growing pigs fed on Acacia tortilis treated with Polyethylene glycol. *Tropical Animal Production, 48, 584-591.*

Hunter, J.E., Zhang, J., Kris-Etheron, P.M. 2010. Cardiovascular disease risk of dietary stearic acid compared with trans, other saturated, and unsaturated fatty acids: a systematic review. *American Journal of Clinical Nutrition, 91 (1), 46-63.*

Indu, M., Ghafoorunissa, P. 1992. *N*-3 fatty acids in Indian diets-Comparison of the effects of precursor (alpha-linolenic acid) *vs.* product (long chain *n*-3 polyunsaturated fatty acids). *Nutrition Research 12,569-582*.

Jiang, J., Xiong, Y. L. 2016. Natural antioxidants as food and feed additives to promote health benefits and quality of meat products: A review. *Meat Science*, *120, 107–117*.

Khanyile, M. Ndou, S.P., Chimonyo, M. 2014. Influence of *Acacia tortilis* leaf meal based diets on growth performance of pigs. *Livestock Science, 167, 211-218.*

Khanyile, M., Ndou, S.P., Chimonyo, M. 2016. Influence of *Acacia tortilis* leaf meal-based diet on serum biochemistry, carcass characteristics and internal organs of finishing pigs. *Animal Production Sci*ence, http:// dx. Doi. Org / 10. 1071/ AN 15182.

Kaur G, Cameron-Smith D, Garg M, Sinclair AJ. 2011. Docosapentaenoic acid (22:5*n*-3): a review of its biological effects. *Progress in Lipid Research 50(1),28-34.*

López, J.A, Chen, J. 2009. "Pathophysiology of venous thrombosis". *Thrombosis Research*, *123, S30-34*.

Mandal, S., Ghosh, K. 2010. Inhibitory effect of Pistia tannin on digestive enzymes of Indian major carps: an in vitro study. *Fish Physiology and Biochemistry*, *36, 1171–1180.*

Mapiye, C., Chimonyo, M., Dzama, K., Hugo, A., Strydom, P.E., Muchenje, V. 2011. Fatty acid composition of beef from Nguni Steers supplemented with *Acacia karroo* leaf-meal. *Journal of Food composition and analysis, 24, 523-528.*

Marume, U., Chimonyo, M., Dzama, K. 2012. Influence of dietary supplementation with *Acacia karroo* on experimental haemocnhosis in indigenous Xhosa lop-eared goats of South Africa. *Livestock Science, 144, 123-139.*

Mas, G., Laval, M., Coll, D., Roca, R., Diaz, I., Gispert, M., Oliver, M.A. Realini, C.E. 2010. Carcass and meat quality characteristics and fatty acid composition of tissues from Pietrain-crossed barrows and gilts fed an elevated monounsaturated fat diet. *Meat Science, 85, 707-714.*

Maw, S. J., Fowler, V. R., Hamilton, M., Petchey, A. M. 2003. Physical characteristics of pig fat and their relation to fatty acids composition, *Meat Science, 63, 185-190.*

Miura, K., Stamler, J., Brown, I.J., Ueshima, H., Nakagawa, H., Sakurai, M., Chan, Q., Appel, L.J., Okayama, A., Okuda, N., Curb, J.D., Rodriguez, B.L., Robertson, C., Zhao, L., Elliot, P. 2013. Relationship of dietary monounsaturated fatty acids to blood pressure: the international study of macro/ micronutrients and blood pressure. *Journal of Hypertension, 31, 1144-1150*.

Mokoboki, H.K., Ndlovu, L.R., Ngambi, J.W., Malatje, M.M., Nikolovav, R.V. 2005. Nutritive value of Acacia tree foliages growing in the Limpopo Province of South Africa. *South African Journal of Animal Science 35 (4), 221-228*.

Mukumbo, F.E., Maphosa, V., Hogo, A., Nkukwane, T.T., Mabusela, T.P., Muchenje, V. 2014. Effect of Moringa Oleifera leaf meal on finisher pig growth performance, meat quality, shelf life and fatty acid composition of pork. *South African Journal of Animal Sc*ience, *44, 388-400.*

Nakamura, M.T., Nara, T.Y. 2004. Structure, function, and dietary regulation of Δ6, Δ5, and Δ9 desaturases. *Annual Review of Nutrition, 24, 345-376.*

Ndou, S.P., Khanyile, M., Chimonyo, M. 2015. Growth performance and nutritio*n*-related serum metabolites in growing pigs fed on *Acacia tortilis* leaf meal. *Livestock Sci*ence, *182, 22-27*.

NRC. 2012. Nutrient Requirements of Swine, 11th rev. ed. Natl Acad. Press. Washington. DC.

Peterson, A. D., Baumgardt, B. R**.** 1971. Food and energy intake of rats fed diets varying in energy concentration and density. *Journal of Nutrition, 101, 1057-1068*.

Purchas, R.W., Knight, T.W., Busboom, J.R. 2005. The effect of production system and age on concentrations of fatty acids in intramuscular fat of the of the longissimus and triceps brachii muscles of Angus-cross heifers. *Meat Science, 70, 215-221.*

Reed, J. D., McDowell, R. E., Van Soest, P.J., Horvath, P.J. 1982. Condensed tannins: a factor limiting the use of cassava forage. *Journal of Science Food and Agriciculture, 33**,** 213- 220.*

Robertson, J.B., Van Soest, P.J. 1977. The fibre estimation in concentrates feedstuffs. In: Proceeding of the 69 Meeting of the American Association of Animal Science. University of Wisconsin, Madison, MI, USA, 23–27 July 1977.

Salter, A. M. 2013. Dietary fatty acids and cardiovascular disease. *Animal, 7(s1), 163-171.*

SAS 2009. SAS User's Guide: Statistics, Version9.1. SASInstitute, Cary, NC, USA.

Simopoulos, A.P. 2008. The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases. *Experimental Biology and Medicine, 233 (6), 674-688.*

Simopoulos, A.P. 2011. Importance of the omega-6/omega-3 balance in health and disease: Evolutionary aspects of diet. *World Review Nutrition Diet*, *102, 10–21*.

Simopoulos, A.P. 2016. An increase in the omega-6/omega-3 fatty acid ratio increases the risk for obesity. *Nutrients, 8(3), 128.*

Smith, S.B., Gill, C.A., Lunt, D.K., Brooks, M.A. 2009. Regulation of fatty acid composition in beef cattle. *Asian-Australian Journal of animal Science*, *22 (9), 1225-1233.*

Swanson, D., Block, R., Mousa, S. A. 2012. Omega-3 Fatty Acids EPA and DHA: health benefits throughout life. Advances in Nutrition: *An International Review Journal 3(1), 1-7.*

Turner, T.D., Mapiye, C., Aalhus, J.L., Beaulieu, A.D., Patience, J.F., Zijlstra, R.T., Dugan, M.E.R. 2014. Flaxseed fed pork: *n*-3 fatty acid enrichment and contribution to dietary recommendations. *Meat Science, 96, 541-547*.

Vahmani, P., Mapiye, C., Prieto, N., Rolland, D.C., McAllister, T.A., Aalhus, J.L., Dugan, M.E.R. 2015. The scope for the manipulating polyunsaturated fatty acid content of beef: A review, *Journal of Animal Science and Biotechnology, 6, 29.*

WHO 2003. Diet, nutrition and the prevention of chronic diseases, Report of a joint WHO/FAO expert consultation, World Health Organization, Geneva, Switzerland, WHO technical report series 916, 2003.

Wood, J. D., Enser, M., Fisher, A. V., Nute, G. R., Richardson, R. I., Sheard, P. R. 1999. Manipulating meat quality and composition. *Proceedings of the Nutrition Society, 58, 1-8.*

Wood, J.D., Richardson, R.I., Nute, G.R., Fisher, A.V., Campo, M.M., Kasapidou, E., Sheard, P.R., Enser, M. 2003. Effects of fatty acids on meat quality: A review. *Meat Science, 66, 21-32*.

Wood, J. D., Richardson, R. I., Nute, G. R., Fisher, A. V., Campo, M. M., Kasapidou, E., Sheard, P. R., Enser, M. 2004. Effects of fatty acids on meat quality: a review, *Meat Science, 66, 21–32.*

Wood J.D., Enser M., Fisher A.V., Nute G.R., Sheard P.R., Richardson R.I., Hughes S.I., Whittington F.M. 2008. Fat deposition, fatty acid composition and meat quality: A review. *Meat Science, 78, 343-358.*

Ulbricht, T.L.V., Southgate, D.A.T. 1991. Coronary heart disease seven dietary factors. *Lancet, 338, 985-992.*

Chapter 7: General Discussion, Conclusions and Recommendations

7.1 General Discussion

The broad objective of the study was to assess relationship between graded levels of V. tortilis leaf and pork quality in general. The main hypothesis tested was that *Vachellia tortilis* leaf meal improve pork quality up to a point where further inclusion would compromise pork quality. Five selected Vachellia species namely. *xanthophloe, V. robusta, V. tortilis, V. nilotica* and *V. nigrescens* were assessed for their protein precipitating activity and antioxidant Forty-eight Large White male pigs with initial body weight of 63 ± 0.93 (SD) kg BW) and 14 weeks of age were fed experimental diets consisting of 0, 30, 60, 90, 120, and 150g/kg V. *tortilis* leaf meal, respectively, for 32 days. Feed intake, average daily gain and feed efficiency ratio were recorded and pigs were slaughtered at the end of experimental period for the determination of carcass characteristics and pork quality.

In Chapter 3, protein precipitating and antioxidant activity of five selected *Vachellia* leaves species were determined. The effect of *V. tortilis* leaf meal on growth and carcass characteristics was determined in chapter 4. Chapter 5 dealt with the effect of physico-chemical attributes of pork from pigs fed on *V. tortilis* leaf meal. Fatty acid profile, atherogenesity, and thrombogenecity indices of pork from pigs fed on *V. tortilis* leaf meal were determined in Chapter 6.

Vachellia tortilis and *V. xanthophloe* had highest protein precipitating capacity. *Vachellia xanthophloe* and *V. robusta* had highest antioxidant activity. All other *Vachellia* species tested were comparable to that of synthetic antioxidant (Ascorbic acid and butylated hydroxyethylene). Protein precipitating capacity and antioxidant activity was affected by specie. The study has shown that *Vachellia* leaves extracts can be exploited as natural source of antioxidant.

Overall, ADFI increased linearly while ADG and G: F ratio decreased linearly with inclusion of *V. tortilis* in Chapter 4. The inclusion of *V. tortilis* linearly decreased slaughter weight, warm carcass weight, cold carcass weight, cooler shrink and back-fat thickness while dressing percentage, pH_{45} and $temperature_{45}$ were not affected by *V. tortilis* inclusion in Chapter 4. The increase in ADFI was attributed to the effect of phenolic compounds such as condense tannins and fiber which limit the possible intake of nutrients there by causing pigs to eat more to compensate for nutrient deficiencies. The study has shown that the increase in leaf meal decreases ADG and G: F ratio, consequently reducing carcass characteristics. This is in agreement with a report by Khanyile *et al*. (2016) who reported a decrease in carcass characteristics of pigs fed on varying levels of V. tortilis leaf meal.

Ultimate meat pH (pH_{24}), in intramuscular fat (IMF), pork redness (a^*) Hue and Warner Bratzler Shear Force (WBSF), increased with increasing levels of *Vachellia tortilis* while moisture, lightness (L^*), yellowness (b^*), Chroma and cooking loss

decrease linearly with increasing *V. tortilis* in the diet. Dry matter percentage and ash of pork were not affected by the diet in Chapter 5. The results are in agreement with the findings by Lee *et al*. (2012), who reported an increase in ultimate pH of thigh meat from broilers fed 1.0% dietary gallic acid and linoleic acid. The quadratic increase in ultimate pH observed in the current study was within the normal range (5.7-6.1), indicating no signs of pale, soft and exudative (PSE) or dark, firm and dry (DFD) meat as described by de Vries and van der Wal, 1992. The quadratic decrease in IMF% can be ascribe to decrease in ADG. The decrease of colour L* was ascribed to higher ultimate pH of pork from pigs fed on *V. tortilis* leaf meal (Bidner *et al.,* 2004). The quadratic decrease in a* was also attributed to the increase in ultimate pH observed with increasing levels of *V. tortilis* leaf meal. Mukumbo *et al*. (2014) also reported a decrease in a* of pork from pigs fed on *Moringa oleifera.* High ultimate pH affect the colour stability of fresh meat by reducing enzyme activity and the rate of oxygenation (Govindarajan, 1973).

The observed linear decrease in cooking loss agrees with the report by Ngambu *et al.* (2013) who reported a decrease in cooking loss of meat from goats supplemented with *V. karroo.* Warner Bratzler Shear Force (WBSF) increase with the increased *V. tortilis* leaf meal. Meat texture largely depends on the size of muscle fibre, amount of connective tissue, and quantity of IMF (Joo *et al.*, 2013). Intramuscular fat is reported to function as lubricants of muscle fibre during cooking, consequently, reducing shear force and increasing the tenderness of meat (Luchak *et al.,* 1998).

In Chapter 6, a study was conducted to determine the effect of *V. tortilis* leaf meal on Fatty acid composition, atherogenesity and thrombogenecity indices of pork. The hypothesis tested was that *V. tortilis* leaf meal has no effect fatty acid, atherogenecity and thrombogenecity indices of pork. The observation that feeing graded levels of *V. tortilis* leaf meal in pigs resulted in a decrease in total fatty acid content could be ascribed to inhibition of lipid synthesis and/or lipid mobilization, resulting from the formation of complexes between proanthocyanidins and enzymes involved in these processes (Aiura and de Carvalho, 2007; Mandal and Ghosh, 2012). The finding that individual and total SFA decreased linearly with increasing levels of *V. tortilis* leaf meal was associated with complexing effect of proanthocyanidins, which have been previously reported to decrease intramuscular fat content (Chapter 5), consequently, decreasing total SFA. High proportion of ALA and LA in the diet could have also resulted in decrease total SFA. High proportion of ALA and LA in the diet are reported to reduce *denovo* synthesis of FA (Duran-Montge *et al*., 2010). Food nutritionist recommends that the intake of thombogenic and atherogenic fatty acids such as myristic (14:0) and palmitic (16:0) in foods including red meat in the human diet be reduced (Salter, 2013; Jiang and Xiong, 2016).

The majority individual and total *n*-6 PUFA decrease linear with increased inclusion levels of *V. tortilis* leaf meal could be partly ascribed to reduced maize and soybean with increase inclusion of *V. tortilis* leaf in the diet. Maize and soybean are reported

to be a major sources of n-6 fatty acid (Kouba *et al*., 2003; Liu, 2015). The level of n-3 PUFA tended to increase with increasing levels of α-linolenic acid. Mapiye *et al*. (2011) reported an increase in α-linolenic acid *n-3* fatty acids in beef fed supplemented with *Vachellia* leaf meal. Low dietary levels of LA or ALA levels in finishing pigs are reported to increase body depositions of LA and ALA (80-100%), while high levels of dietary LA or ALA levels tend to reduce body depositions of LA and ALA by 60-70% (Duran-Montge *et al*., 2010). The inclusion of V. tortilis leaf meal in finishing pig diets reduced atherogenecity index partly through reduction of thombogenic and atherogenic fatty acids such as myristic (14:0) and palmitic (16:0) in foods including red meat in the human diet (Fortin *et al*., 2005; Salter, 2013; Jiang and Xiong, 2016;).

7.2 Conclusions

Results of the study demonstrated that *V. xanthophloe leaf extracts* has high antioxidant activity and that V. robusta, V. nigrescens, and V. tortilis leaf extracts were comparable to that of synthetic antioxidant. *Vachellia* leaf meal could be explored and exploited as a natural source of antioxidant in meat, consequently, improving colour stability and retard lipid oxidation. The inclusion of *V. tortilis* leaf meal in finishing pigs reduce performance, carcass characteristics, intramuscular fat. Pork colour, cooking loos and ultimate pH was improved with increase inclusion levels of *V. tortilis* leaf meal. *V. tortilis* leaf in finishing diet has the beneficial effect in increasing *n*-3 PUFA, monounsaturated fatty acid, nutritive value index and reduce *n*-6: *n*-3 ratio, atherogenic and thrombogenic indices in

pork. The largest proportions of individual and total *n*-3PUFA were between 62 and 70g/kg, DM of *V. tortilis* leaf meal. It can be concluded that Vachellia tortilis leaf meal could be explored in producing organic pork. Omega-3 PUFA such as α-linolenic acid (ALA, 18:3*n*−3) a precursor for EPA, DPA and DHA have probably received the most attention in recent years due to their contribution to human health and well-being. The LC*n*−3 PUFAs, EPA and DHA have a broad range of biological actions involving cell membrane integrity, signal transduction, gene expression, and lipid mediators that together suggest a strong role for prevention of CVD, metabolic and inflammatory diseases and cancer (O'Connell, *et al*., 2017; Wysoczański *et al*., 2016). In addition, DHA has been linked to cognitive and visual development in early life (Dyall, 2015). Based on the beneficial effect of omega -3 fatty acid on human health it is recommended that *Vachellia tortilis* leaf meal be included at rate of 70g/kg DM of feed.

7.3 Recommendations

It is recommended that *V. tortilis* leaf meal in finishing pig diet used as a potential substitute of synthetic antioxidant. Ifquality is the main interest over the reduction of feeding cost, *V. tortilis* leaf meal could be incorporated in finishing pig diet up to 75g/kg DM to increase *n*-3 PUFA and reduce *n*-6: *n*-3 ratio, atherogenetic and thrombogenic indices.

Areas that require further research include:

- Effect of *V. tortilis* leaf on mineral distribution in pork muscles.

- Effect of *V. tortilis* leaf meal on antioxidant stability of pork
- Effect of *V. tortilis* leaf meal on shelf life of pork.
- Sensory evaluation of pork from pigs fed on *V. tortilis* leaf meal
- Ascertain the economic viability of using various levels of *Vachellia tortilis* in pig's diet.

7.4 References

Aiura, F.S., de Carvalho, M.R.B. 2007. Body lipid deposition in Nile tilapia fed on rations containing tannin. *Pesquisa Agropecuária Brasileira. 42, 51-56*.

Bidner, B.S., Ellis, M., Brewer, M.S, Campion, D., Wilson, E.R., Mckeith, F.K. 2004. Effect of ultimate pH on the tenderness of retail meat. *Meat Science, 59, 139-154.*

de Vries, A.G. van der Wal, P.G. 1992. Breeding for quality. In: E Puolane and D.I. Demeyer, (Eds).

Duran-Montge, P., Realine, C.E., Barroeta, A.C., Lizardo, R.G., Esteve-Garcia, E. 2010. De novo fatty acid synthesis and balance of fatty acids of pigs fed different fat sources*. Livestock Science, 132, 157-164.*

Dyall, S. C. 2015. Long-chain omega-3 fatty acids and the brain: A review of the independent and shared effects of EPA, DPA and DHA. *Frontiers in Aging Neuroscience,7, 52.*

Fortin, A., Robertson, W., Tong, A. 2005. The eating quality of Canadian pork and its relationship with intramuscular fat. *Meat Science*, *69, 297-305.*

Govindarajan, S. (1973). Fresh meat colour. *CRC Critical Reviews in Food Technology, 1*,*117-140*.

Jiang, J., Xiong, Y. L. 2016. Natural antioxidants as food and feed additives to promote health benefits and quality of meat products: A review. *Meat Science*, *120, 107-117*.

Joo, S.T., Kim, G.D., Hwang, Y.H., Ryu, Y.C. 2013. Control of fresh meat quality through manipulation of muscle fiber characteristics. *Meat Science, 95, 828-836.*

Khanyile, M., Ndou, S.P., Chimonyo, M. 2016. Influence of *Acacia tortilis* leaf meal-based diet on serum biochemistry, carcass characteristics and internal organs of finishing pigs. *Animal Production Science, http:// dx. Doi. Org / 10. 1071/ AN 15182.*

Kouba, M., Enser, M., Whittington, F. M., Nute, G. R., Wood, J. D. 2003. Effect of a high-linolenic acid diet on lipogenic enzyme activities, fatty acid composition, and meat quality in the growing pigs. *Journal of Animal Science, 818, 1967-1979.*

Lee, K.H., Jung, S., Kim, H.J., Kim II.S., Lee, J.H., Jo, C. 2012. Effect of dietary supplementation of the combination of gallic and linoleic acid in thigh meat of broilers. *Asian-Australian Journal of Animal Science, 25, 1641-1648*.

Liu, Y. 2015. Fatty acids, inflammation and intestinal health in pigs. *Journal of Animal Science and Biotechnology, 6, 4.1*

Luchak, G. L., Miller, R. K., Belk, K. E., Hale, D. S., Michaelsen, S. A., Johnson, D. D., West, R. L., Leak, F. W., Cross, H. R., Savell, J. W. 1998. Determination of sensory, chemical and cooking characteristics of retail beef cuts differing in intramuscular and external fat. *Meat Science, 50, 55-72.*

Mandal, S., Ghosh, K. 2010. Inhibitory effect of Pistia tannin on digestive enzymes of Indian major carps: an in vitro study. *Fish Physiology and Biochemistry*, *36, 1171-1180.*

Mapiye, C., Chimonyo, M., Dzama, K., Hugo, A., Strydom, P.E., Muchenje, V. 2011. Fatty acid composition of beef from Nguni Steers supplemented with *Acacia karroo* leaf-meal. *Journal of Food Composition and Analysis, 24, 523-528.*

Mukumbo, F.E., Maphosa, V., Hogo, A., Nkukwane, T.T., Mabusela, T.P., Muchenje, V. 2014. Effect of Moringa Oleifera leaf meal on finisher pig growth performance, meat quality, shelf life and fatty acid composition of pork. *South African Journal of Animal Sc*ience, *44, 388-400.*

Ngambu, S., Muchenje, V., Marume, U. 2013. Effect of Acacia karroo supplementation on growth, ultimate pH, colour and cooking losses of meat from indigenous Xhosa Lop-eared Goats. *Asian-Australian Journal of Animal Science, 26, 128-133.*

O'Connell, T. D., Block, R. C., Huang, S. P., Shearer, G. C. 2017. ω3-polyunsaturated fatty acids for heart failure: Effects of dose on efficacy and

novel signalling through free fatty acid receptor 4. *Journal of Molecular and Cellular Cardiology, 103, 74-92.*

Salter, A. M. 2013. Dietary fatty acids and cardiovascular disease. *Animal*, 7(s1), 163-171.

Wysoczański, T., Sokoła-Wysoczańska, E., Pękala, J., Lochyński, S., Czyż, K., Bodkowski, R., Librowski, T. 2016. Omega-3 fatty acids and their role in central nervous system – A review. *Current Medicinal Chemistry, 23(8), 816–831.*

Appendix 1. 1 Ethical clearance certificate

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

10 November 2015

Mr Mbongeni Khanyile
School of Agricultural, Earth & Environmental Sciences
Pietermaritzburg Campus

Dear Mr Khanyile,

Protocol reference number: AREC/101/015D
Project title: Fatty acid profile, oxidative stability and quality of pork from pigs fed on *Acacia tortilis* leaf meal-based diets

Full Approval – Research Application

With regards to your revised application received on 03 November 2015. The documents submitted have been accepted by the Animal Research Ethics Committee and **FULL APPROVAL** for the protocol has been granted.

Any alteration/s to the approved research protocol, i.e Title of Project, Location of the Study, Research Approach and Methods must be reviewed and approved through the amendment/modification prior to its implementation. In case you have further queries, please quote the above reference number.

Please note: Research data should be securely stored in the discipline/department for a period of 5 years.

The ethical clearance certificate is only valid for a period of one year from the date of issue. Renewal for the study must be applied for before 10 November 2016.

I take this opportunity of wishing you everything of the best with your study.

Yours faithfully

.....

Dr Shahidul Islam
Chair: Animal Research Ethics Committee

/ms

Cc Superivsor: Dr M Chimonyo
Cc Registrar: Mr Simon Mokoena
Cc NSPCA: Ms Lebo Sentle
Cc Ukulinga Research Farm – Alet Botha

Animal Research Ethics Committee (AREC)
Ms Mariette Snyman (Administrator)
Westville Campus, Govan Mbeki Building
Postal Address: Private Bag X54001, Durban 4000
Telephone: +27 (0) 31 260 8350 **Facsimile:** +27 (0) 31 260 4609 **Email:** animalethics@ukzn.ac.za
Website: http://research.ukzn.ac.za/Research-Ethics/Animal-Ethics.aspx